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AVIAN PARTHENOGENESIS

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FOREWORD

This publication consists of excerpts of articles and the reminiscences of one of our retired senior scientists, Dr. M. W. Olsen, a lead researcher on parthenogenesis in turkeys. The purpose is to describe the efforts involved in the conduct of his work and to document his findings. He describes in his own words the trials and tribulations of conducting biological research, and the personal satisfaction of achieving success. His persistent and productive efforts, borne out by this record, mark him as an exceptionally dedicated research scientist. I am confident that the reader will find the material both informative and interesting.

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AVIAN PARTHENOGENESIS

By Marlow W. Olsen 1/

SUMMARY

Parthenogenesis in birds' eggs is a spontaneous and mostly an abortive form of development. Nucleated cells have been found microscopically in 80 to 90 percent of the blastodisks from newly laid, unfertilized eggs of unselected Beltsville Small White (BSW) and Broad Breasted Bronze (BBB) turkeys. Cells in the disks of newly laid eggs, however, are highly disorganized, moribund, and mostly incapable of normal development once eggs have been placed in the incubator.

This publication pertains primarily to those incubated eggs that have undergone a more advanced type of parthenogenetic development, that is, eggs in which growth of the blastoderm has increased to the extent that it can be detected macroscopically.

Special strains of BSW turkeys and Dark Cornish (DC) chickens have been developed at the Agricultural Research Center, Beltsville, Md. Their unfertilized eggs show a strong predisposition for parthenogenesis. These special strains were developed through selection in the presence of live fowl pox virus. Forty to fifty percent of all unfertilized eggs laid by hens of these special strains can be expected after 10 days' incubation to develop parthenogenetically to a degree that growth of the blastoderms can be detected macroscopically. Effects of such factors as environment, selection, and live avian viruses on parthenogenesis have been discussed.

Parthenogenesis in birds' eggs is an automictic, faculative form of diploid parthenogenesis in which only males appear. More than 1,100 parthenogens have been hatched during this 20-year project.

About 20 percent of the mature parthenogens produced limited quantities of semen containing viable spermatozoa. Semen collected from 26 parthenogens has been used to artificially inseminate virgin turkeys. Viable, healthy poults of both sexes have been sired by each of 26 parthenogens. Sons and daughters of parthenogenetic males have demonstrated their ability to reproduce bisexually.

Unfertilized turkey and chicken eggs and parthenogens have been used in cytological and embryological studies. Moreover, live parthenogens were used in experiments on skin grafting, studies on homozygosity and lethal genes, and in the development of isogenetic lines.

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INTRODUCTION

Parthenogenesis is generally defined as the production of a new individual from an unfertilized egg without the participation of a male or a male gamete. The term is derived from two Greek nouns, "parthenos" meaning virgin and "genesis" to be born, hence the popular terms "virgin birth" or "fatherless individual."

Parthenogenesis implies the birth of a new individual. However, this concept has become greatly modified with usage over the years. Beatty (12) 2/ defined parthenogenesis as "the production of an embryo from a female gamete without genetic contribution from a male gamete, and with or without eventual development into an adult." He further stated, "This deliberately wide definition includes the special case of gynogenesis, in which a spermatozoon does enter the egg and activates it into development, but does not exercise its second role of contributing genetic material." Many scientists today regard any degree of development in unfertilized eggs as parthenogenesis, and some workers even refer to early cleavage as embryos.

Throughout this publication the term "parthenogenesis" refers either to a limited or an abortive type of development or to any subsequent development stage, including formed embryos and hatched parthenogens. On the other hand, the term "embryo" refers to a macroscopically recognizable embryo possessing such structures as somites, limb buds, heart, and head.

CLASSIFICATION

The term "parthenogenesis" is very broad in scope and has been applied to many species, some of which exhibit different types of parthenogenetic development. For clarity and ease in studying and understanding parthenogenesis, it has been classified on the basis of types of parthenogenesis, sex of parthenogens, and cytological events. The following classification is taken from Suomalainen (127).

Types of Parthenogenesis

Occasional or accidental parthenogenesis.--Occasionally unfertilized eggs develop parthenogenetically.

Normal parthenogenesis:

- Obligatory parthenogenesis.--Eggs always develop parthenogenetically.
  - Constant or complete parthenogenesis.--All generations are parthenogenetic.
  - Cyclical parthenogenesis.--One or more parthenogenetic generations alternate with normal bisexual reproduction.
  - Paedogenesis.--The eggs of individuals at larval stage undergo development. This type of parthenogenesis exhibited by gallflies and midges, is closely associated with cyclic parthenogenesis.

2/ Figures in parentheses refer to Literature Cited, p. 80

Faculative parthenogenesis.--An egg may be either fertilized or developed parthenogenetically. Parthenogenesis in chickens and turkeys is of this type.

Sex of Parthenogens

Arrhenotoky.--Unfertilized eggs develop into parthenogenetic males; fertilized eggs in all instances develop into females, e.g., sawflies, honey bees, and wasps.

Thelytoky.--Unfertilized eggs develop into parthenogenetic females, e.g., gall wasps, water fleas, and plant lice.

Deuterotoky.--Unfertilized eggs develop into males or females.

Cytological Events

Generative or haploid parthenogenesis.--Parthenogenetically produced individuals develop from eggs in which the chromosomes have been reduced, resulting in haploid chromosomes, e.g., bees and wasps.

Somatic parthenogenesis.--Parthenogenetic individuals have diploid or polyploid chromosomes.

Automictic parthenogenesis.--The regular chromosomes have been reduced. Subsequently the diploid chromosomes are restored through fusion of two haploid nuclei, the formation of a restitution nucleus, or endomitosis. This type of parthenogenesis corresponds to the ameiotic parthenogenesis of White (134). In automictic parthenogenesis the early state of meiosis is quite normal and is similar to this stage in animals with fertilization. The chromosomes pair at zygotene and form bivalents. As a result of chromosome reduction during meiosis, the zygoid nuclear phase in the eggs becomes haploid. However, the diploid phase is soon restored when the two haploid cells fuse. Animals developing as a result of this type of parthenogenesis always have a diploid soma. This mode of parthenogenetic reproduction is relatively common in animals, e.g., chickens and turkeys.

Apomictic parthenogenesis.--In developing eggs neither chromosome reduction nor nuclei fusion nor any of the preceding corresponding phenomenon occurs in apomictic parthenogenesis.

NATURAL OCCURRING PARTHENOGENESIS

Parthenogenesis is a very common, naturally occurring phenomenon in most animal groups (99, 127, 132, 134). In many species parthenogenesis has become an efficient, normal mode of reproduction. It is found most frequently among invertebrates. At least 90 percent of all known cases of parthenogenesis are estimated to occur among invertebrates, especially insects. However, all members of the same species do not necessarily reproduce in the same manner.

In one geographical location the members of a given species may reproduce exclusively by parthenogenesis, whereas in another location the same species may normally reproduce bisexually. This phenomenon is sometimes referred to as geographical parthenogenesis. It is known in different forms among mollusks, crustaceans, myriapods, and insects and is thought to represent a feature of adaptation (50).

When parthenogenesis occurs in eggs of higher vertebrates, it is usually abortive in nature and very limited--that is, cell divisions are initiated, but for some reason not fully understood, mitosis fails to continue for long. This rule holds true especially for mammals. Naturally occurring parthenogenesis in bird eggs is generally abortive, but there are a few notable exceptions.

Most workers regard parthenogenesis as a variant form of reproduction and relatively recent in origin (99, 127, 132, 134). Each species exhibiting parthenogenetic development is thought to have come originally from ancestors who themselves were reproduced bisexually.

This transition from the bisexual to the parthenogenetic form is believed to indicate selective adaptation to adverse environmental conditions that occurred some time ago. Such factors as quality and availability of food and changes in light and temperature are frequently cited as possible contributing causes of shifts in type of reproduction. Viral infections also may cause shifts in types of reproduction in chickens and turkeys, possibly inducing changes leading to parthenogenetic reproduction. Viral and bacterial infections can be potent mutagenic agents, and mutations induced through pathological conditions might have served to alter the genotype of some species and to cause shifts in their type of reproduction.

Parthenogenesis in mammalian eggs generally continues only for a limited time and seldom goes beyond the cleavage stage. In the eggs of some fishes, amphibians, and reptiles, development proceeds further, and in some instances live parthenogens have been produced. Various aspects of parthenogenetic development in unfertilized eggs of insects, fishes, amphibians, and reptiles have been reported (1, 15, 17, 35, 51, 52, 54, 57, 116, 119, 120).

Bloom 3/ surveyed articles published from 1926 through 1964 on natural parthenogenesis in the animal kingdom. He listed 258 articles according to volumes and specific abstract numbers found in the "Biological Abstracts." He also included in this survey 15 reviews and books written about parthenogenesis.

3/ Bloom, S. B. A review of natural parthenogenesis in the animal kingdom. Master's Thesis, Dept. of Poultry Sci., Pa. State Univ. 39 pp.

Cleaved eggs have been found frequently on the ovaries of several animals. Marshall (50) reviewed literature on parthenogenetic cleavages of ovarian and in unfertilized tubal and uterine eggs in rats, mice, guinea pigs, hamsters, ferrets, vol, rabbits, bats, armadillos, opossums, and man. Likewise Dederer (27) found cleaved ovarian eggs in cats and Olsen (60) in ovaries of domestic chickens and turkeys.

Many of the cleaved mammalian eggs resemble two-, three-, four-, and eight-cell stages, morulae, and even blastocysts. Workers, however, do not agree completely as to the origin of these cleaved eggs. Some believe that cleaved ovarian eggs represent instances in which two or more immature ova when formed become encased within a single follicle (27, 55). Others consider the apparent development to be some aspect of degenerative changes within regressing follicles. Still others consider such development to be early stages of parthenogenesis (46). In many instances the cleaved eggs appear normal in all respects, with no visible indication of existing degenerative changes. They probably have undergone parthenogenetic development at least to some degree.

ARTIFICIAL PARTHENOGENESIS

Some unfertilized eggs of both vertebrates and invertebrates have been activated artificially by various treatments. For example, unfertilized eggs of the silkworm (Bombyz mori L.) will develop parthenogenetically after having been immersed in hot water at 46° C for 18 minutes. This method of activation as reported by Astaurov (2, 3) is referred to as thermal artificial parthenogenesis.

Eggs of sea urchins and starfish have been activated after appropriate treatments in hypotonic and hypertonic salt solutions. Unfertilized eggs of both frogs and toads have been induced to develop by pricking them with a fine glass needle previously dipped in the blood of a foreign species (10, 33, 53, 135).

An abortive type of parthenogenesis has been induced in the unfertilized eggs of several mammals by applying heat or cold or by subjecting them to an electric current. Included are the eggs of mice (31), rats (5), rabbits (22, 100), ferrets (23), and sheep (129, 130). Eggs of mammals, however, usually develop only to an early cleavage stage and generally fail to implant after having been activated and upon being replaced in the tubes of a receptive host.

More success was achieved with mice by using an electric current to stimulate the eggs. This treatment was given in utero, and the eggs were recovered after 7 or 8 days. Two embryos survived for some time following implantation, one reaching the somite stage (128).

Several excellent reviews on artificial parthenogenesis are available in which treatments and methods used to induce parthenogenesis are discussed in greater detail (11, 12, 53, 131, 135).

HISTORICAL REVIEW OF PARTHENOGENESIS IN CHICKEN EGGS

Early investigators found cell-like bodies upon sectioning blastodisks of unfertilized chicken eggs. Some of these structures appeared to contain nuclei. Not all of these early investigators, however, agreed as to whether these cell-like bodies were true cells or whether they represented only terminal stages in the fragmentation of the protoplasmic disks.

Oellacher (56) was the first to report what he considered to be cells of parthenogenetic origin in blastodisks of newly laid unfertilized hens' eggs. He considered them true cells because of their location and their ability to multiply by mitotic division. Duval (29) also observed these cell-like bodies in disks of newly laid unfertilized eggs and like Oellacher (56) considered them to be true cells. Lacaillon (44) reported finding indications of definite parthenogenetic development in freshly laid unfertilized hens' eggs. He prepared 56 drawings of these cell-like structures and in some he showed cells undergoing division. Bartelmez and Riddle (9) working with newly laid unfertilized pigeon eggs found cell-like bodies and considered them to be true cells and of parthenogenetic origin.

Olsen 4/ made a detailed study of blastodisks of unfertilized hens' eggs. Some of the unfertilized eggs examined were obtained prematurely before lay, whereas others were freshly laid. Many nucleated, cell-like bodies found in both groups of eggs were considered true cells and of parthenogenetic origin. Olsen was able to show that cleavages of parthenogenetic origin appeared first in uterine eggs approximately 8 to 10 hours after ovulation. These cell-like structures continued to divide until oviposition, when the greater part of each protoplasmic disk became segmented into hundreds of relatively large yolk-ladened blastomeres. The cells had become so necrotic and disorganized that they could not develop further.

Kosin (39) examined 100 blastodisks from newly laid unfertilized chicken eggs. He also found these cell-like bodies. By using Feulgen stain, he was able to demonstrate chromatin in 15 percent of the nuclei examined. This led him to conclude that some unfertilized chicken eggs undergo an abortive type of parthenogenetic development, but that the process in this instance is of short duration and cannot be revived upon incubation.

4/ Olsen, M. W. Maturation, fertilization and early cleavage in hens' eggs. Egg of the domestic fowl. Ph.D. thesis, Univ. of Maryland, 72 pp. 1941.

On the other hand, Barfurth (7) could not find histological evidence for true parthenogenetic development in sections of blastodisks of the chicken eggs he examined. The cell-like bodies were devoid of nuclei. He concluded that these structures represented only products of fragmentation and were not true cells. Lillie (45, p. 35) reached the same conclusion. He stated, "The so-called parthenogenetic cleavage of such eggs (hen) is merely a phenomenon of fragmentation of the protoplasm; there is no true cell division." Hayes and Nicolaides (36, p. 76) examined sections of blastodisks from freshly laid unfertilized hens' eggs for evidence of parthenogenesis, but found no cell-like bodies in sections they examined. They stated, "Many series of sections from fresh-laid infertile eggs and from infertile eggs incubated for varying periods show no evidence of parthenogenesis. . . The authors are inclined to agree with the large group of workers holding that parthenogenesis does not occur in hens' eggs."

ORIGIN AND NORMAL REPRODUCTIVE CYCLE OF BSW TURKEYS

The origin of the BSW variety of turkeys and various aspects of their normal reproductive cycle is of interest.

The development of the Beltsville Small White (BSW) turkeys was begun in 1934. The main objective was to produce a small compact white bird with the following characteristics: Early maturity, good livability, high egg production, fertility, and hatchability. A considerable number of preexisting varieties of turkeys contributed to the development of the BSW, including the wild turkey, Narragansett, Bronze, White Holland, Black turkey, and a small type of white Austrian turkey. For a complete history of this development, see Marsden (48).

Fertile BSW eggs hatch in about 27 days, approximately 24 hours sooner than eggs of the larger varieties of turkeys. Poults hatched in the late spring usually attain sexual maturity in about 28 to 30 weeks, at which time females weigh about 11 to 12 pounds and males 20 to 22 pounds. Occasionally a young male receiving 14 hours or more of light daily may produce a limited amount of semen as early as 20 weeks. Seventeen weeks was the earliest at which the author obtained traces of semen from precocious young males.

The female turkey invites the mating. The male will not force his attentions on the female as does the male of the domestic chicken. Furthermore, an intact hymen or occulding plate is present in the lower region of the oviduct of immature females. This is a further precaution of nature against premature fertilization.

DISCOVERY OF PARTHENOGENESIS IN TURKEY EGGS

Eggs of mated Beltsville Small White (BSW) turkeys at the Agricultural Research Center, Beltsville, Md., in the winter of 1952 abruptly declined in fertility. Tests were made to determine the cause for loss of fertility.

Males heading the several breeding pens were handled and each was undergoing a general pre-seasonal molt. However, the hens showed no indication of feather loss. On the basis of these findings, Olsen assumed that possibly the loss of feathers from the male birds might be discouraging mating and thus could explain the sudden decline in fertile eggs. To explore this assumption, plans were made to maintain a flock of nonmated BSW turkey females to test the fertilizing ability of semen from molting males. If loss of feathers was discouraging mating, then artificial insemination should theoretically increase the average level of fertility.

A tester flock of 29 nonmated BSW females was isolated from males for 56 days before they were inseminated. In view of the long survival of turkey sperm in the body of the female and the possible chance that some hens had accidently come in contact with males, we tested some of their eggs before using the hens in the proposed experiment. Eggs from these 29 hens were collected and incubated for 7 days before being broken and examined for fertility. By that time, embryonic growth if present can be detected easily either by candling or by breaking the eggs.

When these eggs were broken, a retarded type of embryonic growth was found in a few of them. Repeated tests at weekly intervals for several months showed the same type of embryonic development and at approximately the same weekly level. The last eggs tested were laid 224 days after isolation of the females and still this abortive development persisted. This embryonic growth was mostly abnormal because at least 2 or 3 days of incubation were required before there was any discernible change in the macroscopic appearance and size of the germinal disk. Furthermore, 98 percent of the eggs undergoing development showed no evidence of blood or embryo formation. In spite of the absence of an embryo, cells of the chorio-allantoic membranes continued to grow until thickened sheets of cells covered most of the yolk surface. Of the 934 eggs laid by these 29 nonmated turkey hens, 16.3 percent developed to some degree. In later experiments the same typical 2- to 3-day delay in the growth and enlargement of the blastoderms was noted in unfertilized eggs laid by mated birds (93, 94).

Studies on parthenogenetic development at Beltsville in 1952 and 1953 were essentially exploratory. The following precautionary measures were taken: (1) Insure that all hens involved were virgin and maintained as such throughout the experiment. (2) Insure against mistaken identity of eggs during both their production and incubation. (3) Make certain that the birds were not hermaphoditic. (4) Identify any environmental factor that conceivably might contribute to parthenogenetic development.

Studies in 1953 included 23 virgin BSW females and their unfertilized eggs. Seven of these females were isolated at 4 weeks of age from their immature pen mates. Positive sex identification was made possible by a laparotomy. The other 16 females were brooded together with immature males of their own age until 12 weeks of age. The 16 females were then individually caged with the 7 females until maturity in complete isolation from the males.

BSW turkeys are only about half grown at 12 weeks of age, the females weighing only 4 pounds and the males about 5 1/2 pounds. As previously noted, the earliest age at which semen was obtained from any BSW male was 17 weeks.

Strict precautionary measures were taken to insure against mistaken identity of unfertilized eggs laid by virgin females. During 8 weeks from March 6 to May 10, 1953, the author assumed full responsibility for care of the birds, gathering the eggs, and their incubation. Because groups of mated turkeys were in the same general area, the experimental birds were kept in a locked building and each egg as collected was marked with ink detectable only under ultraviolet light.

Olsen and Marsden explored the possibility that the turkey, like some fishes, might be hermaphroditic (122), that is, females producing both sperm and eggs. The late Mary Juhn studied and interpreted ovaries of young turkeys of various ages. Because Dr. Juhn detected no testicular tissue, Olsen and Marsden concluded that sperm were not involved in the abortive type of development observed in unfertilized turkey eggs.

An attempt was made during 1953 to identify certain environmental factors that might be associated with parthenogenesis. Such factors as location of the experimental flock with respect to other turkeys, feed, light, and sex hormones were checked. None of these factors were found to be closely associated with parthenogenesis. Because sperm were definitely not involved, the author concluded that the development first found in 1952 was a form of naturally occurring, abortive parthenogenesis in eggs of higher vertebrates.

PARTHENOGENESIS IN MATED TURKEYS

With the discovery of parthenogenesis in unfertilized turkey eggs in 1952, studies were undertaken to determine whether this phenomenon was related to infertility. Before this question could be fully resolved, however, it was necessary to establish (1) that parthenogenetic development does occur in unfertilized eggs of mated turkeys and (2) that parthenogenesis is initiated at a stage of egg formation when it might interfere with the successful union of male and female pronuclei.

Experiments were designed to establish whether unfertilized eggs from mated flocks would develop parthenogenetically. The first experiment conducted by Olsen and Marsden (94) produced only circumstantial evidence that parthenogenetic development does occur among unfertilized eggs of mated turkeys. In this study, eggs laid by mated BSW turkeys were incubated for 24 to 72 hours and then segregated by candling into two classes, fertilized and unfertilized. The unfertilized eggs were then further incubated. Among 675 eggs that still appeared clear before the candler at 72 hours of incubation, 151 or 18.2 percent on further incubation developed embryonic tissue but without embryos. Olsen and Marsden suggested that this abortive development in apparently unfertilized eggs was of parthenogenetic origin, a view not fully shared by Kosin (40).

In a second experiment, a genetic color marker was used to resolve the question of parthenogenesis in eggs of mated turkeys. This method of approach involved mating recessive white turkey females, selected for a high incidence of parthenogenetic embryos, to Dark Cornish (DC) chicken and to New Jersey Buff (NJB) turkey males. Thus a marker gene was incorporated in the mating, which served to establish by down color, parentage of embryos surviving for at least 15 days within the shells. Furthermore, the sex of individual embryos judged to be parthenogenetic served as still another marker, because Poole and Olsen (106) showed that all parthenogens were males.

Semen from DC chickens was used to inseminate 148 BSW turkey hens. These hens represented a strain selected for a high incidence of parthenogenetic embryos. An additional 16 selected BSW turkey hens were inseminated with partly inactivated semen from homozygous NJB turkey males that had been held 1 hour in the freezing compartment of a household refrigerator. Plumage color of both DC chickens and NJB turkeys is dominant to the recessive white of BSW turkeys.

After insemination of BSW turkey hens with untreated semen from DC chickens, 105 embryos 15 days old or older were obtained. This included 17 embryos and 3 poults, all males and with white down, indicating that they had developed from unfertilized eggs. This parthenogenetic development occurred even though viable chicken sperm were presumably present in the infundibulum.

After insemination of the 16 BSW hens with partly inactivated (chilled) semen from homozygous NJB males, 22 brown poults comprised of both sexes, 1 white male poult, and 2 white male embryos were obtained from 124 turkey eggs. The white parthenogenetic male was helped from the shell and survived for 12 weeks. The two white parthenogenetic embryos attained the size of normal 26- to 27-day turkey embryos. Results of this second experiment show that unfertilized eggs from flocks of mated turkeys can develop parthenogenetically when viable sperm fail to penetrate the ovum. It is well established therefore that parthenogenesis can and does occur in unfertilized eggs of mated turkeys (69).

The next important question is whether a conflict exists between normal and parthenogenetic development. Based on cytological studies and related data, Olsen (76) concluded that although both parthenogenetic development and normal fertilization are initiated in the infundibulum and are closely related, there is no direct conflict between them.

When viable sperm are present and penetrate the ovum, meiosis II is completed, permitting fusion of haploid sperm and egg pronuclei. When viable sperm are absent or when sperm fail to penetrate the ovum, extrusion of the second polar body is probably not completed. Chromosomes destined for the second polar body and those of the egg nucleus probably never completely separate. They eventually fuse and form a reconstituted nucleus containing the diploid number of chromosomes. Subsequently such an unfertilized ovum resumes development as a diploid cell with all genetic material of maternal origin.

INCIDENCE OF PARTHENOGENESIS IN TURKEY AND CHICKEN EGGS

An abortive type of parthenogenesis that could be revived by incubation was demonstrated for the first time in 1952 in eggs of BSW turkeys and then in 1953 in infertile eggs of DC chickens. Since these original observations, the unfertilized eggs of many breeds, varieties, and strains of fowl have been incubated. A general survey was made of the frequency of parthenogenesis. Data in tables 1 and 2 were obtained by Olsen (72) and Olsen and Marsden (95).

Table 1.--Incidence of parthenogenetic development in unfertilized eggs of standardbred and crossbred chickens, Beltsville, Md.

Breed, strain, or cross	Hens	Eggs	Parthenogenetic development
	Number	Number	Percent
Araucana	25	310	0
Barred Plymouth Rock	51	1,245	.16
Old English Black Breasted Game	7	114	0
Dark Cornish, Beltsville	375	15,629	6.38
Commercial A	12	754	.66
Commercial B	41	1,314	.38
Silver Cornish, Beltsville	42	3,407	2.26
New Hampshire, Beltsville	29	795	0
Rhode Island Red, Beltsville:			
Outbred	19	232	.43
Inbred	94	2,107	0
White Leghorn:			
Outbred, Beltsville	38	606	0
Outbred, Commercial	87	984	0
Inbred, Beltsville	94	1,911	.05
Inbred, East Lansing	169	2,430	.12
Crosses:			
Dark Cornish, Commercial A, X Beltsville	11	510	3.92
Dark Cornish, Beltsville, X New Hampshire	56	1,444	1.18
New Hampshire X Silver Cornish	77	1,703	.88
New Hampshire, Commercial, X Barred Plymouth Rock, Commercial	30	444	0

Table 2.--Incidence of parthenogenesis in unfertilized eggs of various strains and varieties of domestic turkeys, Beltsville, Md.

Strains, varieties, and cross	Hens observed	Hens producing parthenogenetic development	Eggs examined	Total parthenogenetic development	Embryos
	Number	Number	Number	Percent	Number
Thompson Broad Breasted White	11	10	281	22.4	2
Empire Broad Breasted White	11	8	253	8.7	2
New Jersey Buff	18	14	579	6.9	2
Broad Breasted Large Bronze (Univ. of California)	9	2	100	8.0	0
Broad Breasted Large Bronze (Giant Strain)	15	8	299	13.0	0
Broad Breasted Large Brown	6	5	235	4.3	0
Broad Breasted Large Brown X Broad Breasted Bronze	5	3	168	3.6	0
Light Palm	11	9	199	13.1	0
Beltsville Small White	30	28	934	16.9	2
Total	116	87	3,048	12.2	8

Crittenden and Wilcox (pers. comm.) incubated 2,430 unfertilized eggs of White Leghorn (WL) chickens from their inbred lines 6, 9, 10, 14, and 151 in tests for parthenogenetic development. The eggs were incubated for 8 to 10 days before being broken and classified macroscopically for parthenogenetic development. Only three eggs developed parthenogenetically and three eggs were classified as questionable.

Kosin, Sato, and Nagra (43) also tested unfertilized eggs of Washington State White turkeys and those of a variety of chicken known as Olympia. Approximately 8 percent of 367 unfertilized turkey eggs and 0.59 percent of 1,022 unfertilized chicken eggs incubated for 7 days developed to some extent.

E. G. Buss, Pennsylvania State University, likewise determined the incidence of parthenogenesis in unfertilized eggs of a considerable number of unselected turkeys. The eggs were incubated for 10 days before being broken and examined macroscopically for development. Unpublished data from this university are given in table 3.

Table 3.--Incidence of parthenogenetic development in unfertilized eggs of various varieties, reciprocal F_1's, and an inbred turkey, University Park, Pa.

Variety or cross		Hens	Eggs	Parthenogenetic development
Sire	Dam			
		Number	Number	Percent
Bronze	Bronze	43	866	5.0
Black-Winged Bronze	do	45	820	3.8
Bronze	Black-Winged Bronze	43	957	2.0
Black-Winged Bronze	do	36	526	2.3
Bronze	Bronze	43	866	5.0
Black	do	17	319	4.4
Bronze	Black	17	346	13.3
Black	do	15	271	14.8
Bronze	Bronze	43	866	5.0
Gray	do	18	405	2.5
Bronze	Gray	19	395	.5
Gray	do	19	381	.5
Inbred-Bronze	Black-Winged Bronze	19	379	6.3
Do	Black	15	308	15.6
Do	Grey	19	405	1.5
Do	Bronze	16	315	14.6
Do	Inbred-Bronze	18	241	19.9
Total		445	8,666	5.6

The significance of findings at University Park was the relatively low average incidence of parthenogenetic development in unfertilized eggs and the complete absence of parthenogenetic embryos. In contrast, at Beltsville parthenogenetic embryos were found in eggs of four of the nine unselected commercial strains and varieties of turkeys examined (table 2).

The method of determining macroscopically the incidence of parthenogenesis in eggs after a given period of incubation is not completely accurate. It gives only those instances in which cells of parthenogenetic origin at time of lay are still sufficiently viable so that they can be revived upon incubation. A more accurate method of determining the incidence of parthenogenesis can be obtained by sectioning blastodisks from newly laid unfertilized eggs. This method has been followed by several workers. Average levels of parthenogenesis determined on the basis of numbers of nucleated embryonic cells in sectioned blastodisks are considerably higher than levels determined in unfertilized eggs incubated for 10 days.

Kosin and Nagra (41) found nucleated cells in 80 percent of unfertilized sectioned blastodisks from newly laid eggs of Broad Breasted Bronze (BBB) turkeys. Haney and Olsen (34) found nucleated cells in 90 percent of the sectioned blastodisks from unfertilized eggs of unselected BSW turkeys. Normally only 16 to 18 percent parthenogenesis is found in intact unselected BSW turkey eggs after incubation for 10 days.

Poole and Olsen (106) compared the two methods of classifying unfertilized eggs for parthenogenesis--microscopic examination at lay and macroscopic examination after 10 days' incubation. Unfertilized eggs of three strains of DC hens were examined. Blastodisks of some newly laid unfertilized eggs were sectioned and examined microscopically for the presence of nucleated cells. Other unfertilized eggs from the same hens were classified macroscopically for parthenogenesis after 9 to 10 days' incubation. Their data revealed that the incidence of parthenogenesis varied greatly in eggs from different strains. Invariably the incidence of parthenogenesis was much higher when eggs were classified microscopically based on the presence or absence of nucleated cells than when classified macroscopically after incubation (table 4).

Table 4.--Incidence of parthenogenetic development in unfertilized eggs of 3 strains of Dark Cornish chickens based on birds and eggs when observed microscopically at laying and macroscopically after incubation, Beltsville, Md.

Strain	At laying		After incubation	
	Eggs	Parthenogenetic development	Birds or eggs	Parthenogenetic development
	Number	Percent	Number	Percent
		INCIDENCE IN BIRDS		
A----------	11	100	11	63.60
B----------	12	83	12	33.30
C----------	41	56	41	7.30
Total or average--	64	69	64	21.90
		INCIDENCE IN EGGS		
A----------	22	100	510	3.90
B----------	24	66	754	.66
C----------	82	43	1,314	.38
Total or average--	128	57	2,478	1.65

Source: Poole, H. K., and Olsen, M. W. (106).

Based on the data by Poole and Olsen (106) we can assume that a high proportion of all unfertilized eggs of chickens and turkeys probably undergo a spontaneous, abortive type of parthenogenesis. In most instances this development is detectable only upon microscopic examination of disks from newly laid eggs. Most cells in newly laid unfertilized eggs are inviable. This means that other eggs from the same hens after 10 days' incubation may not develop to the degree that embryonic growth can be detectable by macroscopic examination.

CHARACTERISTICS OF EARLY PARTHENOGENESIS

Before Lay

Parthenogenetic development in unfertilized eggs of fowl must be considered as mostly abortive and in most instances as a highly unorganized type of growth. Only rarely can cells in newly laid unfertilized eggs be revived upon incubation (39, 95, 106).

Haney and Olsen (34) showed that first mitotic cell divisions in unfertilized turkey eggs occur at about the time ova enter the uterus. First cleavage in unfertilized turkey eggs is generally delayed about 2 hours when compared with first cleavage in fertilized turkey eggs. Furthermore, the location and angle of the first cleavage plane in fertilized turkey eggs and in eggs developing parthenogenetically may not always bisect each developing blastodisk the same. Bartelmez (8) established that the point of entrance of the sperm in pigeon eggs was definitely related to future orientation of the developing embryo. In parthenogenesis the sperm are not involved and therefore lack organization.

In addition to being delayed, cleavage in parthenogenetic eggs definitely lacks cellular organization. Parthenogenetic cleavage once initiated proceeds at a near normal rate; however, cells tend to fuse, pile up, and form in multiple layers rather than spread laterally as a single-layered blastoderm. Disks have been found in which as many as 10 layers of cells were visible in cross sections with no indication of a segmentation cavity.

Still another characteristic of early parthenogenetic development in uterine eggs is that cells which seemingly form do not differentiate into the small epithelial-type cells found in newly laid fertilized eggs. Parthenogenetic cells, even at time of lay, still consist almost solely of the large yolk-ladened blastomere variety found only during early cleavage in fertilized chicken and turkey eggs (34, 93). Differences in appearance of cells developing in newly laid unfertilized and fertilized turkey blastodisks appear in figure 1.

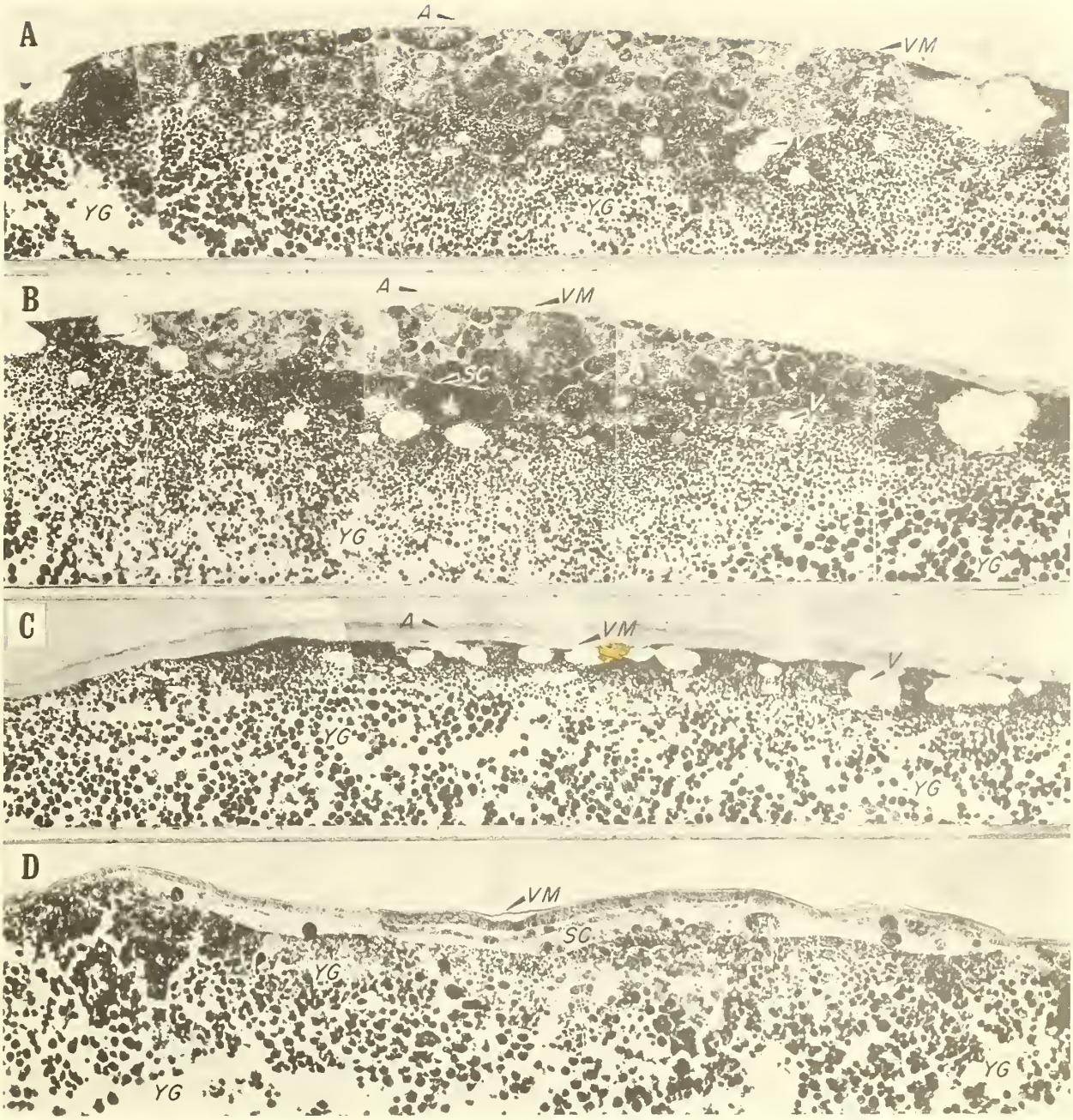

Figure 1.--Median cross sections of blastodisks of four newly laid unincubated turkey eggs (X 150): A and B, Infertile disk showing parthenogentic development from eggs laid by hen 7614. Note depth of proliferating disk, absence of well-defined segmentation cavity (SC), numerous vacuoles (V); and vitelline membrane (VM) with its adhering layer of albumen (A) at upper surface of each disk. C, Infertile disk showing no parthenogenetic development from another egg laid by hen 7614, with no cleavage in disk. Note numerous vacuoles in disintegrating protoplasm. D, Fertile disk taken from egg of hen in contact with sexually mature male. Note absence of vacuoles, limited depth of developing disk, and well-defined segmentation cavity.

Reasons for the failure of cells of parthenogenetic origin of uterine eggs to transform into smaller epithelial-type cells characteristic of the species have not been established.

Still another characteristic of unfertilized uterine eggs is that segmentation generally does not involve the entire protoplasmic blastodisk. Sizable sections of a disk may remain unsegmented, whereas other areas of a disk may have undergone extensive mitotic activity. Furthermore, each protoplasmic disk usually shows unmistakable morbidity by time of lay as shown by the presence of vacuoles and the loss of staining ability on the part of many existing cells. Characteristic differences between cells in fertile and unfertilized eggs are evident in the four photographs of figure 1.

During Incubation

Embryonic development in unfertilized turkey and chicken eggs is delayed by about 2 to 3 days after eggs are placed in the incubator. This delay is believed to represent the time required for disorganized masses of embryonic cells to reorganize and form a normal blastoderm. Although the delay in development was noted previously (34, 39, 92, 93) only three systematic studies (42, 70, 136) have been made of blastodisks during the first few days of incubation. The gross structure and cellular arrangement of unorganized membranes from unfertilized turkey eggs after 7 days of incubation have been described by Olsen and Marsden (92), Yao and Olsen (136), Kosin and Sato (70), and Mun 5/.

Olsen (70) made a detailed study on the gross appearances and cytological events in incubated unfertilized turkey eggs during the first 4 days of incubation. Unfertilized eggs from a strain of BSW turkeys selected for a high incidence of parthenogenesis were examined. Data were collected on the duration of the time lag in onset of development. Two approaches were followed. One was to break a series of unfertilized eggs at various times during incubation and determine the diameter of the developing blastoderms. The second approach was to candle groups of unfertilized eggs at various intervals during incubation and determine the earliest time at which development could be detected.

After 24, 48, 72, 96, 120, and 144 hours of incubation, 100 eggs each were removed and broken. The diameter of each developing blastoderm was measured in millimeters. The results are presented in table 5.

5/ Mun, A. M., Olsen, M. W., and Sarvella, P. Embryo morphogenesis in unfertilized turkey eggs. Developmental Biology. Paper presented at the American Association for the Advancement of Science, Chicago, Ill. 1972.

Table 5.--Number of blastoderms of different sizes found in unfertilized turkey eggs at various hours after incubation, Beltsville, Md.

Incubation hours	Blastoderms with indicated diameter (mm.)				
	3-5.9	5-9.9	10-14.9	15-19.9	20+
	Number	Number	Number	Number	Number
24--------	1	0	0	0	0
48--------	30	5	0	0	0
72--------	8	17	1	1	0
96--------	7	15	2	4	16
120-------	7	3	1	1	29
144-------	1	0	1	0	25

In the candling tests 756 unfertilized eggs were candled after 24, 48, 72, 96, 120, 144, 168, and 240 hours of incubation. At each candling, those eggs in which development could be detected for the first time were identified and returned to the incubator. Eggs in which development could not be detected at the first candling were recandled at intervals of 24 hours until development, if any, was finally detected. The final candling of all eggs was after 10 days of incubation or a 240-hour interval, at which time all eggs except those with living embryos were broken and examined macroscopically.

Of the 756 eggs candled, 312 or 41.3 percent developed to some degree; 215 of these 312 eggs undergoing development or 68.9 percent contained only sheets of unorganized cells. The other 97 eggs had formed embryos. Cumulative percentages of eggs undergoing development at successive 24-hour periods are shown in figure 2, together with data collected in the same manner using fertilized turkey eggs. These data show the comparative extent of the time lag in the development of unfertilized and fertilized turkey eggs.

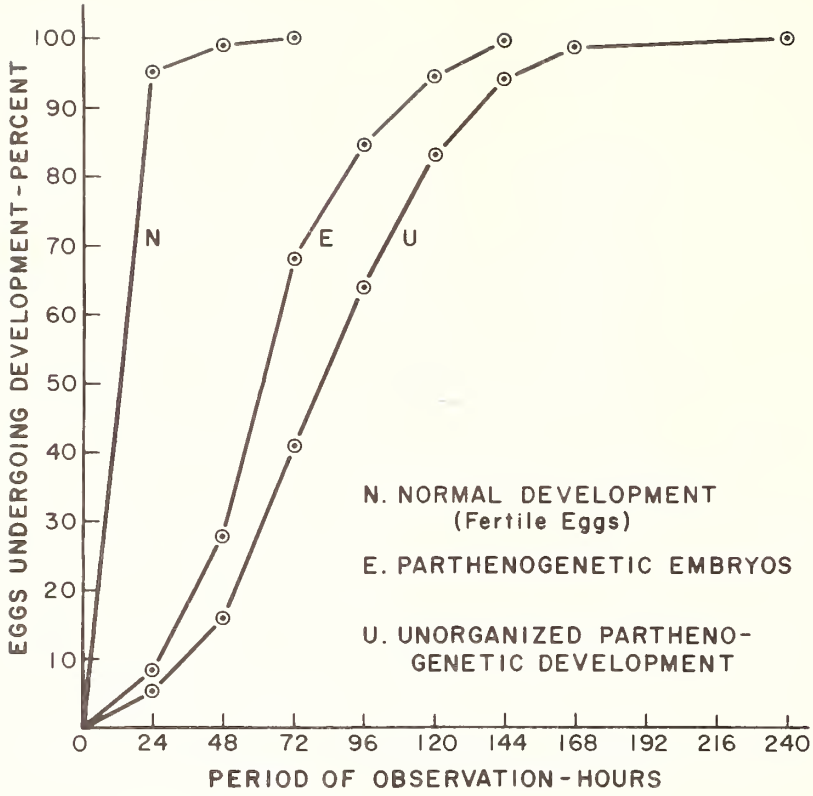

Figure 2.--Cumulative percentages of developing blastoderms of fertilized and unfertilized turkey eggs detected by candling after various incubation periods. (Data for normally developing fertile eggs (N) taken from Olsen and Marsden (94).)

CELLULAR ORGANIZATION WITHIN BLASTODISKS OF FERTILIZED AND UNFERTILIZED TURKEY EGGS

Cleavage in birds' eggs is discordal, involving only the small protoplasmic disk of the ovum. During the early cleavage stages (2- to 16-cell) the large blastomeres are not completely surrounded by cell membranes. They are relatively large segments of protoplasm simply delineated by cleavage furrows. Each of these large blastomeres contains a single nucleus separated from an adjacent nucleus so that it has its own sphere of influence. Subsequent cleavage of these blastomeres, as pointed out by Rugh (110) "...divides the protoplasm independently of the yolk, into progressively smaller units that eventually give rise to the multicellular embryo and its extra-embryonic structures."

By the time of lay all cells of fertilized turkey eggs have been transformed so that they are no longer the large yolk-ladened blastomere type but are rather more of an epithelial cell type. These cells are small and uniform in size and shape, have distinct cell membranes, and reproduce by mitotic divisions. The blastoderm, which has formed by the time of lay, is only one or two cell layers in depth with a distinct segmentation cavity and initiated gastrulation.

The cells found in blastodisks of newly laid unfertilized eggs contrast markedly with those in fertilized eggs. Most cells present are large yolk-ladened blastomeres, and most remain in this primitive state throughout the sojourn of the egg in the oviduct. These blastomeres vary greatly in both size and shape with little or no indication of distinct cell membranes. Embedded within each blastomere, however, is a nucleus. Hundreds of these blastomeres are present in many blastoderms of newly laid, unfertilized turkey eggs. This is the condition of the blastoderm when unfertilized turkey eggs are placed in the incubator.

This atypical cellular arrangement persists in varying degrees throughout the first 4 days of incubation. Cytological studies were made of sections of blastodisks of unfertilized turkey eggs at 24-hour intervals during the first 4 days of incubation. No indication was found that any of the large primitive-type blastomeres underwent true mitotic division.

Figure 3 shows several large yolk-ladened blastomeres and a layer of smaller newly formed epithelial-type cells. Although nuclei in two of the blastomeres appear to be dividing, the cytoplasm surrounding them remains uncleaved. Daughter nuclei seem to have arisen within the intact blastomere and will eventually emerge, as yolk granules composing the greater portion of the blastomere, become disassociated, and disperse into the surrounding protoplasm. This means that the transition from the blastomere to the epithelial-type cell in unfertilized turkey eggs is not simply by repeated divisions of existing cells but rather in one division and after the unfertilized eggs have been placed in the incubator. This method of cell formation suggests the possibility that the cytoplasm composing these relatively large blastomeres may have only a nutritive or "nurse cell" function.

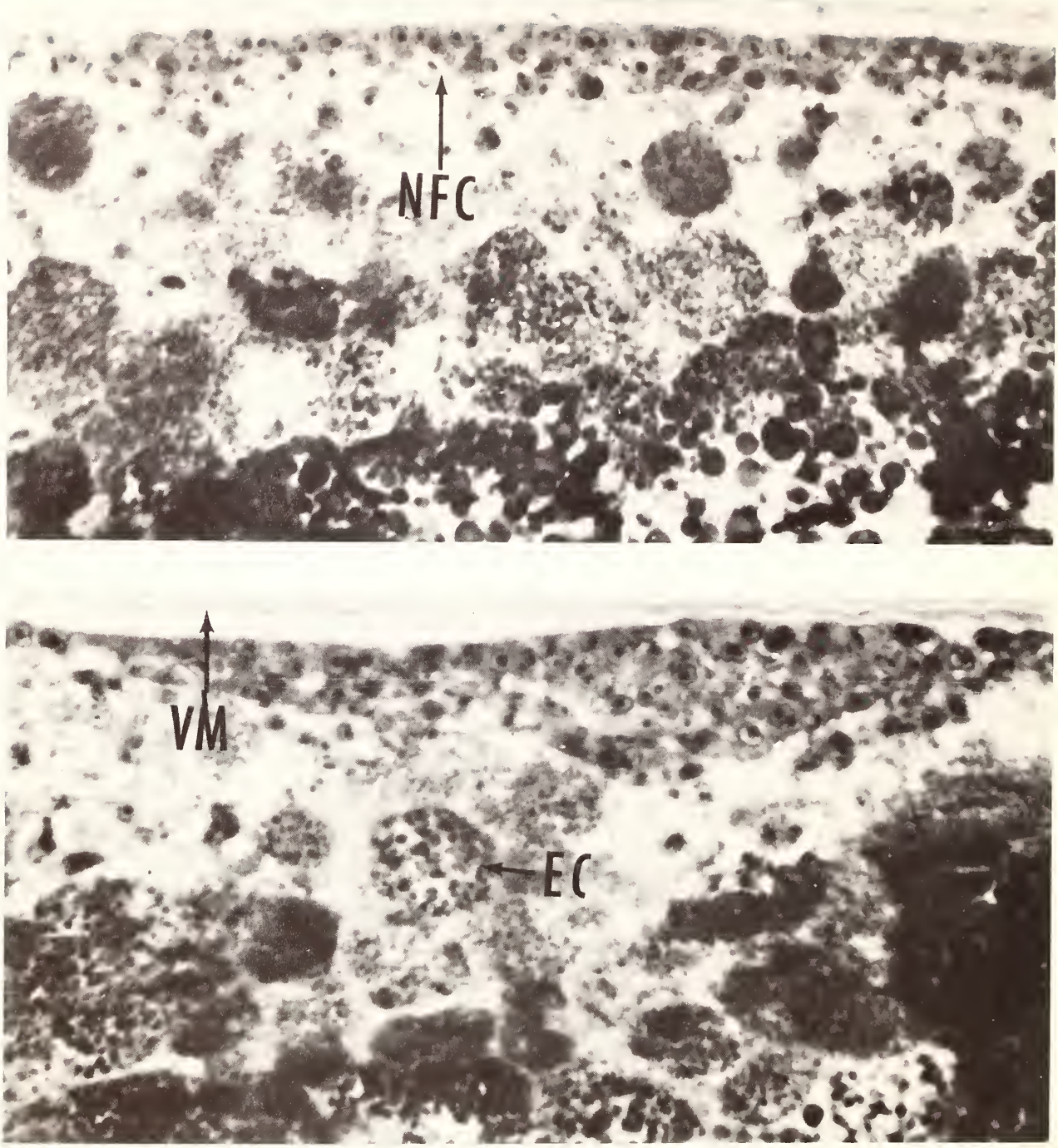

Figure 3.--Median cross sections of blastoderms of two unfertilized turkey eggs after 72 and 96 hours of incubation showing formation of small epithelial-type cells (NFC). These cells have become arranged in multilayers along upper surface of each disk and along underside of vitelline membrane (VM). Note large, yolk-ladened blastomeres (EC) immediately beneath each of these multiple layers of epithelial-type cells. Some blastomeres shown are still intact, whereas others appear to be disintegrating. One blastomere at bottom is dividing (X 680.)

In all preparations studied epithelial-type cells formed both in the lower depths of the blastodisks as well as along their upper surface. Evidently epithelial-type cells as they are formed tend to migrate upward and eventually come to lie just beneath and along the underside of the vitelline membrane. These membranes in such instances serve as a wall along which cells are able to aline themselves and form a single-layered normal-type blastoderm.

Epithelial-type cells after their formation in parthenogenetically developing turkey blastoderms also tend to form in multiple layers (figure 3, bottom). When these cells form in multiple layers, a normal blastoderm is not formed; yet in some instances cells in these thickened layers continue to divide giving rise to thickened sheets of epithelial-type cells that cover most of the yolk surface. These sheets of cells are usually devoid of both blood and embryos.

This transformation and reorganization of embryonic cells in unfertilized eggs taking place within the incubator must be carried out under environmental and temperature conditions quite different from those existing in the hens' oviduct. This may be one reason why less than one-half of the unfertilized turkey eggs known at time of lay to contain nucleated cells resume development on being incubated. Parthenogenetic embryos develop in only approximately 20 percent of the unfertilized eggs that undergo further development within the incubator. Although the transitions from the large blastomeres to the epithelial-type cells occur, usually cells are unsuccessful in organizing themselves into single-cell layers to form normal blastoderms. Therefore we can conclude that the developmental potential of unfertilized eggs, even at best, is low.

Chicken-Turkey Hybrids

An unsuccessful attempt was made to enhance cellular organization in unfertilized turkey eggs and thus to increase chances of obtaining greater numbers of viable parthenogenetic embryos. It was theorized that possibly one factor contributing to the unorganized cellular condition might be the lack of sperm with their organizing effect. Bartelmez (8) demonstrated that some relationship exists between the point of penetration of the sperm and the location of the first cleavage furrow in pigeons' eggs. It was further theorized that possibly some foreign sperm would supply the organizing effect desired in the turkey eggs and yet not contribute any of its own genetic material.

DC and Rhode Island Red (RIR) chicken males were selected as semen donors for BSW turkey females for the following reasons: (1) Chickens and turkeys are from widely separated families of fowl, and 12 previous unsuccessful attempts at hybridization had been made at various experimental stations (32) and (2) should fertilization occur, plumage color of the chicken males being dominant to that of the BSW turkey would serve as genetic markers.

The first inseminations using chicken sperm were made at Beltsville in 1959. This study included 176 virgin and nonmated BSW hens and 2,132 of their eggs. The results obtained had not been anticipated. A total of 120 embryos survived within the shell for 15 days or longer, an age when down color of embryos becomes detectable. This number included 23 embryos that survived to hatching and were helped from the shell. These 120 embryos and poults were colored, indicating that each was of hybrid origin. Furthermore, there was no noticeable increase in numbers of parthenogenetic embryos. This unexpected development, however, led to a series of studies on various aspects of chicken-turkey hybridization (14, 64, 66, 82, 102, 107).

The first hybrid was hatched in May 1959. Four of twenty-three hybrids that hatched in 1959 survived for 12 to 44 weeks. In 1959-60 more than 30 hybrids were hatched. Of these, two of eight birds that hatched in 1960 survived for almost a year, and a third was still alive at 18 months of age and weighed 10.5 pounds. An adult chicken-turkey hybrid is shown in figure 4.

Figure 4.--Three-year-old chicken turkey hybrid weighing 10 pounds (June 1972).

Both the hatched and unhatched chicken-turkey hybrids have characteristics of both parents. Each has the color markings, the short heavy legs, and the fully feathered neck of their chicken sires but the white skin and the long tail feathers of their turkey dams. All hybrids have had flattened, cushion-type combs minus spikes, and all appendages such as snoods, wattles, and ear lobes were missing. All chicken-turkey hybrids are males but are incapable of siring offspring. They make no sound except when frightened.

The gonads of hybrids at hatching are unusually large; they are more than twice the size of those of newly hatched chicks and poults. Poole (102) determined the number of chromosomes in somatic cells of hybrids and found them to have 15 macrochromosomes, which contrasts with 18 in turkeys and 12 in chickens. Since approximately 100 BSW turkey eggs have to be incubated to obtain 1 hatched hybrid (64), no commercial value of hybrids is foreseen. As might be expected, hybrids tend to be malformed and they never mature sexually. Some hybrids at hatching have cross beaks, notched upper beaks, and curled tongues. All young hybrids have required assistance in hatching and had to be hand-raised and forced fed. Incubation time is approximately 23 days, about midway between that of DC chickens and BSW turkeys.

ORIENTATION OF PARTHENOGENETIC EMBRYOS

6 Days of Incubation

Orientation of parthenogenetic embryos is also greatly affected by their near-random mode of origin. Normally a young avian embryo developing within a fertilized egg has a fixed head-tail relationship with respect to the long axis of the shell. When the blunt end of a fertilized egg that is incubated for a few days is held to the left of the observer and the upper part of the shell removed, the embryo usually will be found lying on its right side with its head turned to the right and pointing away from the observer.

Bartelmez (8) found the actual angle between the long axis of the embryo and that of the egg to vary considerably in pigeon eggs. Although the axis angle varies almost 180°, 85 percent of the angles are between 45° and 90°.

Sometimes the heads of young parthenogenetic embryos are directed toward the observer when the egg is held with its blunt end to the left. Embryos thus oriented are termed "inversions." Embryos occupying this position on the yolk are found in about 1 percent of pigeon eggs and in 11.8 percent of fertilized chicken eggs (89).

Orientation of normal embryos in fertilized turkey eggs appears to vary more than that in pigeon and chicken eggs. In spite of this greater variability among turkey embryos, there is still a definite fixed head-tail relationship of most embryos to the long axis of the egg. Orientation of parthenogenetic turkey embryos varies even more than normal embryos of other birds. Their orientation tends to be random.

Considered as normal were 326 or 66 percent of 487 4-day-old normal turkey embryos found in positions on the yolk. In contrast, only 98 or 44.3 percent of 221 6-day-old parthenogenetic turkey embryos were found in the same position. (The longer incubation for unfertilized turkey eggs was to compensate partly for the 2-day time lag in onset of parthenogenetic development.) Completely reversed on the yolk, with their heads pointing toward the observer, were 58 or 11.9 percent of 487 normal turkey embryos. In contrast, 66 or 29.8 percent of the 221 parthenogenetic embryos were found in this reversed position.

The near-random distribution of parthenogenetic embryo positions indicates that there is no predetermined orientation of the future parthenogenetic embryo when the unfertilized ovum leaves the ovary. This orientation is evident, however, in normal embryos from fertilized eggs. Data also indicate the impossibility of predicting with any degree of accuracy which part of the early developing blastoderm will develop eventually into the head end of the future parthenogenetic embryo. Thus a problem arises for the cytologist who may wish to study early stages of parthenogenetic development.

Hatching Stage

Disorientation of 6-day parthenogenetic embryos is also reflected at the time of hatching in lowered hatchability. This is due partly to an increased incidence of malposition. Normally developing embryos, when properly oriented within the shell near hatching time, have their heads at the blunt end of the egg next to the air cell. When they have assumed this normal position, their head and neck are bent to the right and their beaks lie beneath the right wing. Some normal embryos, however, take other positions that are disadvantageous with respect to pipping. Commonly occurring malpositions include head in small end of the shell, head between legs, and head under left wing. Parthenogenetic embryos, which are weak, are especially handicapped in these three malpositions. These parthenogenetic poults rarely succeed in hatching and few of them ever pip.

Olsen and Byerly (89) studied the incidence of these three malpositions in relation to improper orientation of early normal chicken embryos. They established a positive relationship between the high incidence of early embryos with improper early orientation and the incidence of malpositions.

Olsen (77) studied the incidence of the three malpositions among 279 full-term BSW parthenogenetic embryos that had failed to hatch. Approximately 50 percent of these embryos had their heads at the small end of the shell. In contrast, Insko and Martin (38) found only 2.66 percent of the embryos of standardbred Bronze in this position.

About 11.5 percent of the 279 parthenogenetic embryos had their heads under their left wing. This percentage is about 14 times higher than that (0.82 percent) given by Insko and Martin (38) for embryos from fertilized standardbred Bronze eggs. On the other hand, the incidence of parthenogenetic

embryos with their heads between legs was about the same for the standardbred Bronze as for parthenogenetic BSW turkey embryos.

GENETIC AND ENVIRONMENTAL FACTORS ASSOCIATED WITH PARTHENOGENETIC DEVELOPMENT

Inheritance and Its Effect

Stalker (123) studied the inheritance of parthenogenesis in flies Drosophila parthenogeneta and D. polymorpha. He reported that a high degree of heritability exists. Through selection, the incidence of parthenogenesis was greatly increased in eggs of both species. Over a period of 17 years, the rate of viable parthenogenetic progeny of D. parthenogeneta was increased from the original level of 8 to 151 per 10,000 unfertilized eggs. During the same period, the rate of viable parthenogenetic progeny of D. polymorpha increased from 1 to 151 per 19,000.

Carson (18, 19) studied the heritability of parthenogenesis in the fly D. mercatorum. He started with three wild strains having very low rates of thelytokous parthenogenesis; the highest was only about 1 adult female per 1,000 unfertilized eggs. Through selection, the rate in a strain from El Salvador increased tenfold; the rate in a strain from Rochester, N.Y., increased only one-fifthfold from 39 per 1 million to 0.2 percent. Crosses between these two strains produced hybrids with parthenogenetic levels of 6 percent. Selection within an Oahu, Hawaii, strain resulted in a level between 1.5 and 2 percent. These results indicate that certain genes are involved in controlling parthenogenesis and that different strains vary in their predisposition for parthenogenesis.

Corresponding rates of increased parthenogenesis were obtained in selective breeding programs with BSW turkeys and with DC chickens. Parthenogenesis in BSW turkeys increased from the original level of 16.7 percent in 1953 to 41.5 percent in 1959 (62). This represents about a threefold increase in total parthenogenesis in five generations. The most marked increase in unfertilized turkey eggs, however, came in the relative numbers of parthenogenetic embryos. The level increased from 3 per 1,463 (0.2 percent) in eggs of the original stock in 1953 to 342 per 2,929 (11.7 percent) in 1959. This is about a fifty-eightfold increase in the level of parthenogenetic embryos (62, 71). Yearly increases and decreases in levels of parthenogenesis of various categories between 1952 and 1963 are shown in figure 5.

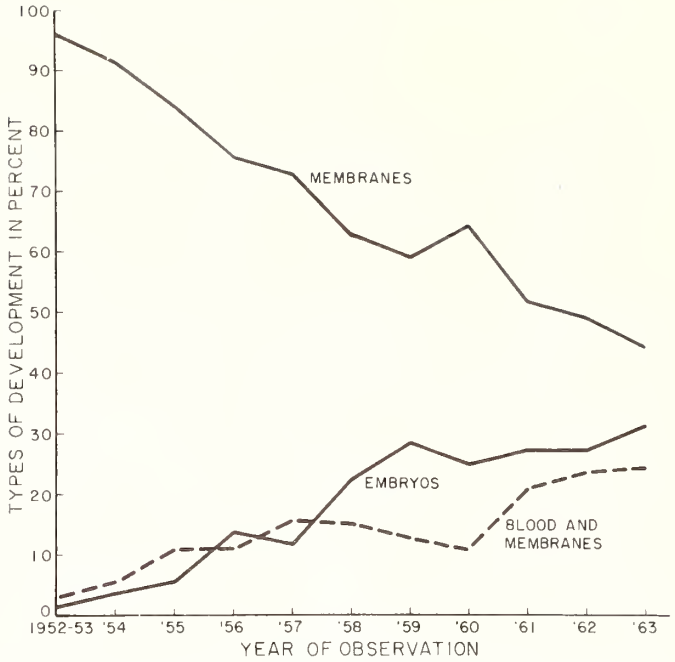

Figure 5.--Incidence of parthenogenetic development in eggs of virgin Beltsville Small White turkeys. Percentages are based on total eggs undergoing parthenogenetic development.

Increases in levels of macroscopically detectable parthenogenesis in unfertilized eggs of Pozo Gray turkeys were likewise obtained at Pennsylvania State University through selection. In a study of five generations of birds, the incidence of unorganized membranes increased from 5 per 474 (1.1 percent) in 1962 to 117 per 627 (18.6 percent) in 1966. This represents an increase of about seventeenfold (87). Selection at Pennsylvania State University did not result in a strain of birds capable of producing parthenogenetic embryos.

Through selection, marked increases in levels of macroscopically observable parthenogenesis were also obtained in eggs of the strain of DC chickens maintained at Beltsville. Parthenogenetic development in eggs of DC chickens generally is not as advanced as in eggs of BSW turkeys. Development consisted primarily of a limited growth of the extraembryonic membranes that are usually devoid of both blood and embryos (Olsen, unpub. data). The parthenogenetic trait in the Beltsville strain of DC chickens has been intensified through years of selection to where 2.1 percent of the unfertilized eggs now yield embryos (112). Five live chicken parthenogens were helped from the shell in 1972 and three of them are still alive (Sarvella, pers. commun.). Olsen, Wilson, and Marks (97) postulated that a single locus, autosomal recessive gene controls parthenogenesis.

Further evidence that some sort of genetic factor exerts a controlling effect on parthenogenesis was obtained at the Agricultural Research Center, Beltsville, Md. Repeatedly when hens whose eggs showed a high incidence of parthenogenesis were crossed with nonparthenogenetic strains of birds,

the resulting progeny produced unfertilized eggs having a percentage of parthenogenetic development that was intermediate with respect to the two parental strains. The modified nature of this effect in such a cross is evident regardless of which way the cross is made.

For example, when DC chicken hens whose unfertilized eggs averaged 5.7 percent parthenogenesis were mated to White Leghorn (WL) males of a non-parthenogenetic line, unfertilized eggs of the resulting female progeny averaged less than 1 percent parthenogenesis (73). Another example was when WL female 9243, with a record of 37.5 percent parthenogenesis, was mated to a DC male from a strain averaging about 7 percent parthenogenesis. Each of eight daughters sired by the DC male produced some eggs that underwent a limited unorganized type of parthenogenetic development, varying from 13.7 to 57 percent. The average incidence of parthenogenesis of the eight daughters from this breed cross was 32.7 percent, a value only slightly lower than that found in unfertilized eggs of their dam (73). One would have expected this value to have been slightly lower and closer to the average level shown by purebred DC.

The reverse situation, however, was found in the level of parthenogenesis among eggs of the second generation of hens. In this instance, six crossbred daughters of WL dam 9243 were mated to purebred DC males. Thirty-nine virgin daughters from these matings laid 1,201 unfertilized eggs, 11.2 percent of which developed parthenogenetically to some degree--an average value approaching the level exhibited by the DC stock (73).

In crosses between high and low parthenogenetic lines of turkeys, the intermediate effect is evident not only in the level of parthenogenesis but also in both viability and in numbers of parthenogenetic embryos (Olsen, unpub. data).

Further evidence for the existence of differences among chickens was disclosed in a general survey of unfertilized chicken eggs of eight breeds and four types of crosses. No predisposition to parthenogenesis was found upon incubating unfertilized eggs of Araucanas, New Hampshires, and Game Chickens. Only 7 of 9,515 eggs laid by WL, Barred Rocks, and RIR developed parthenogenetically. DC chickens (72) of the strain maintained at Beltsville produced unfertilized eggs showing the highest incidence of parthenogenesis (6.38 percent).

A similar survey for parthenogenesis was made of unfertilized turkey eggs, representing those of eight unselected strains and varieties. In contrast to eggs of chickens, eggs of all eight varieties of turkeys showed some degree of parthenogenesis, varying from 3.6 to 22.4 percent of the total eggs examined (95). Thus we can conclude that turkey eggs, in general, are more predisposed to parthenogenetic development than are those of chickens. However, in these two surveys only eggs that had undergone 10 days of incubation were classified. As already noted, the estimate of the level of parthenogenesis in unfertilized eggs is much higher and more accurate when data are collected by microscopically examining cross sections of blastodisks of newly laid eggs for nucleated cells (106).

Environmental Factors

Investigators do not agree completely as to what environmental factors have contributed in past centuries to the origin of parthenogenesis in animals. Such factors as scarcity of food, quality of food, and changes in day length have been suggested as affecting parthenogenetic development in insects. Early literature on the effects of these factors on parthenogenesis in aphids, rotifers, and cladocera was reviewed by Shull (118). Temperature, another environmental factor frequently cited, certainly has affected the development of unfertilized eggs, especially those of insects and other invertebrates. For example, Astaurov (2) demonstrated that parthenogenetic development can be initiated by immersing silkworm eggs in water at 46° C for 18 minutes. Stalker (123) also found that by raising the environmental temperature slightly, he could increase by threefold the incidence of naturally occurring parthenogenesis in eggs of Drosophila parthenogeneta. Bergerard (13) found that he could reverse the sex of parthenogenetic stick insects by raising the environmental temperature during their early development. Eggs incubated at 30° during the first third of embryonic development, with controls incubated at 23°, produced males almost exclusively.

Low temperatures have been used routinely by investigators to activate unfertilized eggs of both vertebrates and invertebrates. An abrupt change in temperature has been very effective in inducing eggs of such animals as rats, mice, ferrets, and rabbits to undergo early stages of development (5, 12, 22, 131). However, whether temperature alone in nature could shift the reproductive pattern of hundreds of species from bisexual to parthenogenetic reproduction is questionable.

Studies were undertaken at Beltsville in 1953 to determine what factors were responsible for parthenogenetic development in eggs of chickens and turkeys. Among those considered were possible hormonel stimuli generated through mating, location of the females with respect to male turkeys, and type of feed. Each of these factors was considered and eventually eliminated as a possible cause of parthenogenetic development.

For example, in one experiment homozygous Bronze turkey males were vasectomized and then allowed to mate with virgin BSW turkeys to determine whether mating as such affected the level of parthenogenesis in their eggs. By using homozygous Bronze males, a genetic color marker was introduced so that the origin of any advanced turkey embryo or poult could be established by down color. Parthenogenesis was not increased in eggs of virgin hens mating with vasectomized males.

Feed was readily discounted as a factor inducing parthenogenesis since chicken hens of certain breeds getting the same feed as turkeys showed little predisposition to parthenogenesis.

Age as a Factor

Another factor that has a pronounced effect on parthenogenesis is the age of the bird. The effect in this instance is not so much on the level of total parthenogenesis but on the character of development. Young virgin turkey hens produced during their first laying season a higher average

percentage of unfertilized eggs that gave rise to parthenogenetic embryos than during their second laying season. Data in table 6 are based on the incubation and classification for parthenogenesis of more than 13,000 unfertilized BSW eggs (75).

Listed separately by years are percentages of parthenogenesis recorded for eggs laid by the same turkey hens during their first and second laying season. In each of the six yearly tests, the percentage of formed embryos was much higher in eggs produced during the first laying season. Despite the greatly increased incidence of embryos over the 9- to 10-year period, the ratio between first and second season eggs remained about the same. The embryos in eggs of these younger birds survived, on the average, longer within the shell, 11.3 days as compared with 7.8 days. These findings suggest that certain physiological changes associated with age tend to inhibit the full expression of parthenogenetic development. The exact reason for the decline in parthenogenetic embryo production the second season is not clear. Possibly the same factors responsible for lowered reproductive performance in older birds generally may also be operative in parthenogenetic embryos.

Season as a Factor

Since the average percentage of parthenogenetic embryos in unfertilized eggs of the same birds was higher during their first laying season than during their second, the question might also be asked, Do unfertilized eggs produced by the same birds during the early part of the testing season differ from those laid during the last part of the same season? To answer this question, records of two groups of young virgin turkeys--one group produced in 1967 and the other in 1968--were analyzed. Data collected during February, March, and April of both years were arranged to show average percentages of total parthenogenesis and parthenogenetic embryos by weeks (figure 6).

The average weekly percent of both total parthenogenesis and parthenogenetic embryos continued rather uniformly throughout each of the two 3-month testing seasons (78).

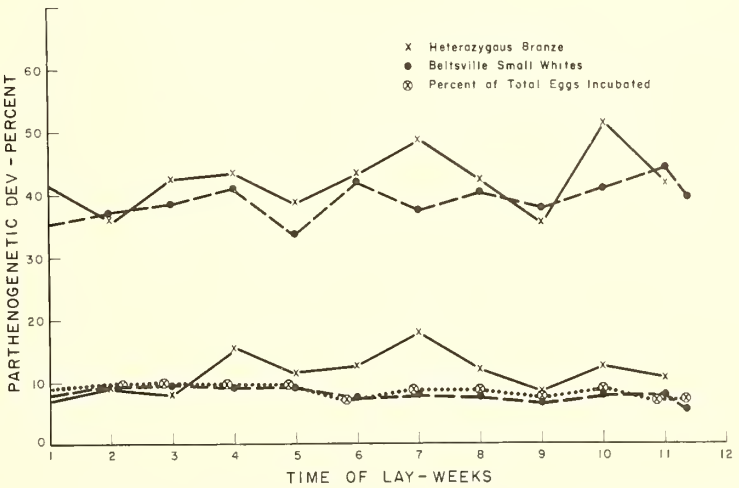

Figure 6.--Average percentage of eggs showing total parthenogenesis and parthenogenetic embryos.

Table 6.--Incidence of parthenogenetic development in unfertilized eggs of Beltsville Small White turkeys during first and second laying seasons, Beltsville, Md.

Laying season	Hens	Eggs	Eggs containing			Parthenogenetic development	Average age of embryos
			Membrane only	Blood and membranes	Embryos		
	Number	Number	Percent	Percent	Percent	Percent	Days
1954----------	20	1,138	30.7	6.4	3.0	40.2	9.1
1955----------	20	738	27.8	2.6	.7	31.0	3.8
1955----------	29	1,534	36.4	10.6	4.2	51.2	8.4
1956----------	29	893	28.7	4.3	.6	33.5	3.0
1956----------	28	1,049	25.4	7.4	10.7	43.6	13.1
1957----------	28	955	38.3	5.2	2.5	46.1	10.7
1957----------	44	1,921	26.6	6.1	7.9	40.6	12.0
1958----------	44	1,104	31.8	5.1	4.6	41.6	8.0
1964----------	31	1,408	19.3	10.4	14.5	44.2	13.9
1965----------	31	1,233	26.2	13.3	4.8	44.3	11.5
1965----------	11	700	17.3	12.3	18.6	48.1	11.5
1966----------	11	418	26.3	16.0	4.8	47.1	10.1
Total or average:							
1st laying season--	163	7,750	26.0	8.9	9.8	44.6	11.3
2nd laying season--	163	5,341	29.8	7.8	3.0	40.6	7.8

EFFECT OF VIRUSES ON PARTHENOGENESIS

Dark Cornish Chickens - Fowl Pox

Dark Cornish (DC) chickens were tested in 1955 and 1956 because their unfertilized eggs were the only ones showing any significant amount of parthenogenesis that could be detected macroscopically (72). Parthenogenetic development in DC eggs consisted typically of a limited growth of embryonic membranes. The following tests with DC chickens were conducted.

In 1955 all eggs of 42 virgin, nonvaccinated DC pullets, laid from January 19 to May 4, were incubated and examined for parthenogenesis. On May 5 these same 42 pullets were vaccinated--13 with live pigeon pox virus and 29 with live fowl pox virus. All eggs laid by these 42 females during the next 4 months were also examined for parthenogenesis. The method of testing was the same--an initial 9- to 10-day period of incubation, after which the eggs were broken and germinal disks examined macroscopically for parthenogenetic development. In 1956 the eggs of 35 DC females were classified for parthenogenesis before and after vaccination with a vaccine containing a live fowl pox virus on April 1. The results of the 1955 and 1956 tests are presented in table 7.

These data reveal that in 1955 more than three times as much parthenogenesis was found in eggs laid after vaccination with live fowl pox virus vaccine than in eggs laid by the same birds before vaccination. ($\underline{X}^2 < 33$, degrees of freedom = 1, $\underline{P} < 0.001$). The milder pigeon pox virus was less effective in inducing parthenogenesis ($\underline{X}^2 < 1$, degrees of freedom = 1, $\underline{P} < 0.35$).

The 1956 tests with live fowl pox virus showed even greater differences--more than nine times the incidence of parthenogenesis in eggs of the same birds after than before vaccination ($\underline{X}^2 = 168$, degrees of freedom = 1, $\underline{P} < 0.001$). The highest incidence of parthenogenesis was in eggs laid 30 to 60 days after the birds were vaccinated.

Facilities were not available for maintaining nonvaccinated birds during the entire tests. However, data on the incidence of parthenogenesis in turkeys do not show any appreciative seasonal variation (78, 92).

Table 7.--Incidence of parthenogenetic development in unfertilized eggs of Dark Cornish chickens before and after vaccination with pigeon pox and fowl pox viruses, Beltsville, Md.

Year and treatment	Before vaccination			After vaccination		
	Hens	Eggs	Parthenogenetic development	Hens	Eggs	Parthenogenetic development
	Number	Number	Percent	Number	Number	Percent
1955						
Chickens vaccinated with--						
Pigeon pox virus----	13	418	1.9	13	666	2.9
Fowl pox virus------	29	1,020	.7	29	1,226	2.4
1956						
Chickens vaccinated with fowl pox virus---	39	1,294	1.62	39	1,139	15.2

A severe disease outbreak, diagnosed as visceral lymphomatosis, occurred among two groups of White Leghorn (WL) chicks when they were approximately 5 to 6 weeks old. Heavy mortality continued throughout the growing period. When these birds reached maturity, unfertilized eggs of the surviving females were tested to see whether this severe viral infection had induced any changes in the potentiality for parthenogenesis noticeably low in eggs of Beltsville strains of WL (73).

Unfertilized eggs tested after 9 to 10 days of incubation showed that four of the hens were producing a significant number of unfertilized eggs that developed parthenogenetically. This finding led to the decision to make a series of matings to determine whether the seemingly virus-associated trait could be transmitted to progeny. A second objective of these matings was to determine whether types of sires would affect significantly the incidence of parthenogenesis in eggs of their virgin daughters.

From February 1 through March 12, 1961, 1,053 eggs were laid by the 37 purebred WL that had survived the leukosis epidemic. The 20 hens derived from stock of another station laid 648 of these eggs and the 17 surviving hens of Beltsville origin laid 405 eggs. No evidence of parthenogenetic development could be detected in the 648 eggs of the imported stock. On the other hand, 17 (4.2 percent) of the 405 eggs laid by 4 of the 17 surviving WL birds of Beltsville origin developed parthenogenetically. The average incidence of parthenogenetic development in eggs of these four hens was 15.1 percent. Of the eggs produced by WL hen 9243, 37.5 percent developed parthenogenetically.

After completion of parthenogenetic tests in 1961, hen 9243 was mated to a DC male. Each of this hen's eight daughters sired by the DC male produced some unfertilized eggs that underwent a limited, unorganized type of parthenogenetic development, varying from 13.7 to 57 percent. The average incidence was 32.7 percent, a value only slightly lower then that of their dam. The average level of parthenogenesis in unfertilized eggs of the DC strain from which the male was derived was slightly less than 7.2 percent of total eggs incubated.

Based on the performance of DC, one would have expected the average level of parthenogenesis in the eggs of the eight crossbred daughters to have been slightly lower and closer to the average value of the DC stock. However, individual males and females have been observed repeatedly to vary greatly with respect to their ability to produce offspring whose eggs develop parthenogenetically.

After 1962 parthenogenetic tests were completed, and six surviving crossbred daughters from WL dam 9243 were mated to another DC male to produce a second generation consisting of 39 virgin females. The average level of parthenogenetic development in their eggs was 11.2 percent, which was slightly closer to the average value of DC eggs. The same six dams later in the 1962 season were remated, this time to two WL males and they produced 13 females.

Only 0.2 percent of their unfertilized eggs developed parthenogenetically. In this instance, the two WL sires depressed the predisposition to parthenogenesis in eggs of their daughters.

This breeding system was continued for several generations. The results are given in table 8.

Turkeys - Fowl Pox Virus

Several studies have been conducted with turkeys in which chick embryo-propagated, live fowl pox virus has been tested for its effect on the level of parthenogenesis in eggs of unselected Beltsville Small White (BSW) turkey females. Because of the lack of adequate isolation facilities, some of the studies had to include tests for levels of parthenogenesis in eggs of the same hens before they were vaccinated for fowl pox and then for a given period after vaccination. In most instances, commercially produced live fowl pox vaccines were used.

Test 1

The first test was conducted in 1956 and included 65 unselected BSW virgin turkey females. The incubation and examination of 3,110 eggs extended for 3 months. The 65 birds were separated from their immature pen mates at 4 weeks of age. They were in two groups--16 nonvaccinated turkeys and 49 turkeys vaccinated for fowl pox at 7 weeks and again at 30 weeks of age. Because the ancestry of each bird was known at the onset of the test, the birds could be selected so that in each group full sisters would be represented. The 16 nonvaccinated turkeys representing 12 families were housed in wire cages in a screened building where they could be isolated from vaccinated birds. The 49 vaccinated birds, representing the same 12 families, were kept in unheated houses where they were in direct contact with other vaccinated birds.

The 16 nonvaccinated turkeys produced 738 unfertilized eggs during the 3-month period. Of these, 180 eggs (24.3 percent) developed parthenogenetically during 9 to 10 days of incubation. Of the 738 eggs, 19 contained well-formed embryos.

The 49 vaccinated females produced 2,362 eggs during the same period. Of these, 750 (31.8 percent) developed parthenogenetically. Well-formed embryos were in 102 of these eggs. The records of these two groups of full sisters reveal that a significantly higher percentage of parthenogenesis and well-formed embryos occurred in eggs laid by the vaccinated group of turkeys (31.8 percent) as compared with 24.4 percent of total eggs tested ($\underline{X}^2 < 12.3$, degrees of freedom = 1, $\underline{P} < 0.001$). Formed embryos were found in 2.6 percent of those laid before vaccination and in 4.8 percent of those laid after vaccination (61).

Table 8.--Incidence of parthenogenetic development in unfertilized eggs of White Leghorn (WL) hen 9243, a survivor of a severe outbreak of visceral lymphomatosis, in eggs of this hen's decendents, and in eggs of Dark Cornish (DC) hens, Beltsville, Md.

Year and group	Parental stock				Progeny			
	Sire	Breed	Dam 1/	Breed or cross 2/	Daughters 1/	Breed or cross 2/	Eggs	Parthenogenetic development
	Number		Number		Number		Number	Percent
1960:								
Experimental--	--	WL	--	WL	1 (hen 9243)	WL	24	37.5
Control-------	--	DC	--	DC	26	DC	341	5.0
1961:								
Experimental--	1	DC	1 (hen 9243)	WL	8	G_1	525	32.7
Control-------	1	DC	6	DC	22	DC	795	7.2
1962:								
Experimental--	2	WL	6	G_1	13	G_2	475	.2
Do--------	2	DC	6	G_1	39	G_2	1,201	11.2
Control-------	3	DC	6	DC	13	DC	437	5.9
1963:								
Experimental--	20	WL	27	G_2	69	G_3	3,423	.7
Do--------	5	DC	27	G_2	42	G_3	2,161	15.0
Control-------	4	DC	7	DC	15	DC	435	5.8
1964:								
Experimental--	15	WL	24	G_3	86	G_4	3,181	2.0
Do--------	15	DC	24	G_3	82	G_4	2,103	14.1
Control-------	5	DC	11	DC	22	DC	625	18.9
1965:								
Experimental--	19	WL	27	G_4	87	G_5	3,171	.56
Do--------	9	DC	27	G_4	61	G_5	1,267	19.7
Control-------	7	DC	12	DC	31	DC	706	17.2

1/ WL hen 9243 and her progeny.

2/ Subscripts indicate number of generations (G) descendents are removed from hen 9243.

Test 2

In the second test of the effectiveness of fowl pox virus as a parthenogenetic-associated agent were two similar groups of unselected BSW turkey females, all of which had been vaccinated for fowl pox when 6 to 8 weeks old. The two groups included a total of 50 birds--23 turkeys that were revaccinated for fowl pox when approximately 36 weeks old and the controls that were not revaccinated. The twice-vaccinated turkeys and the controls produced 1,091 and 1,099 unfertilized eggs, respectively, during the 90-day test period. Approximately 28 percent of the eggs from the twice-vaccinated group and about 17 percent of those from the controls developed parthenogenetically during 10 days of incubation. The eggs of the former had 24 (2.7 percent) embryos and the latter had 4 (0.36 percent). Differences in levels of parthenogenesis in eggs of twice-vaccinated and control turkeys are shown in figure 7 (96).

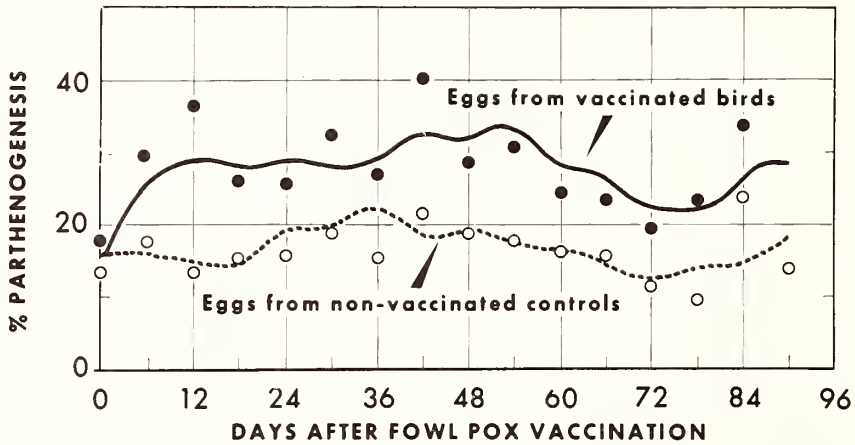

Figure 7.--Percent parthenogenesis in unfertilized eggs of Beltsville Small White turkeys in relation to time of fowl pox vaccination. Hens of treated and control groups were vaccinated for fowl pox when 6 to 8 weeks old and treated hens again after 30 weeks.

Test 3

The third test with the fowl pox virus as a parthenogenetic-associated agent was initiated on January 31, 1961. In this test 19 heterozygous Bronze turkey females (BSW x wild) were transferred to an isolated poultry building and vaccinated for fowl pox. These 19 crossbred hens had been vaccinated for fowl pox when 6 to 8 weeks old and again in October 1960. The vaccine for this third vaccination was a quail-adapted strain of fowl pox prepared by Frank Rauscher of the National Cancer Institute, Bethesda, Md. Eggs laid by these 19 crossbred hens during January 1961 before the third vaccination and from February 1 to March 24, 1961, after the third vaccination were incubated and tested for parthenogenesis. Results from these tests were as follows:

	Eggs	Eggs showing development	Eggs with embryos
	Number	Percent	Percent
Before vaccination--	409	34.5	0.97
After vaccination---	502	36.6	3.38

These data show an average increase in parthenogenesis of 2.1 percent over that in eggs laid by the same hens before vaccination. The increased number of parthenogenetic embryos after vaccination accounts for most of this 2.1-percent difference. In the 409 eggs laid by hens before vaccination, only four embryos (0.97 percent) were found. In the 502 eggs laid by hens after vaccination, 17 embryos (3.4 percent), including one set of triplets, were found.

Vaccination also affected the increased survival time of embryos. The four embryos in eggs laid before the hens were vaccinated grew to the average size of 3- to 4-day-old normal embryos. In eggs laid after the hens were vaccinated, 5 of the 17 embryos grew to the size of 24- to 28-day-old normal embryos. Two of the five parthenogens hatched and one poult was still alive at approximately 2 months of age.

Even though these 19 crossbred hens had been vaccinated for fowl pox before this test at 6 to 8 weeks of age and again in October 1960, some degree of stimulation followed the third vaccination, which was given approximately 4 months after the second one (Olsen, unpub. data).

Test 4

A cooperative study to obtain further information on the respective roles of genetic factors and fowl pox virus in parthenogenesis was undertaken in 1961 with the Pennsylvania State University, University Park. At this university fowl pox vaccine is not used to vaccinate turkeys, whereas at Beltsville it is used routinely each year to inoculate 6- to 8-week-old turkey poults.

Pennsylvania State University provided mature nonvaccinated turkey females, which were pedigreed birds and consisted of 16 full sister pairs of Pozo Gray (PG) turkeys. This variety was selected because it had been identified as having a low incidence of parthenogenesis, 1 to 2 percent. One member of each pair of full sisters (16 birds) was taken to Beltsville when the females were 24 weeks old and vaccinated for fowl pox. The other 16 birds were retained at Pennsylvania State University to serve as nonvaccinated controls. The same males were used at both locations the first year and sisters at both locations were mated to the same male the first year.

Housing and management conditions were standardized as far as practicable so as to minimize environmental effects at each station. Turkeys at Beltsville

were kept in cages during this test. Turkeys at Pennsylvania State University were kept in cages during the first year, but thereafter they were kept in floor pens. Birds at both locations received 14 hours of light daily beginning each year on January 1, when the virgin females were approximately 28 to 32 weeks old.

Eggs were collected daily and placed each evening in forced-draft incubators of the same make. The incubators at both stations were operated at 37.5° C and a relative humidity of 57 percent. Each year after completion of the parthenogenetic tests, certain virgin PG hens were mated to PG males to obtain the poults for the next generation. Each year at each station matings were made to intensify the parthenogenetic trait.

In 1962 at Beltsville, 11 of the 16 vaccinated hens produced one or more eggs showing parthenogenetic development, whereas at Pennsylvania State University, 5 of the 16 nonvaccinated hens produced one or more eggs showing development. The incidence at Beltsville ranged from 0 to 15.1 percent and at Pennsylvania State University from 0 to 3.8 percent. Annual results obtained during 1962-66 at both stations are summarized in table 9.

It is most significant that each year at Beltsville the birds produced unfertilized eggs with a consistently higher level of parthenogenesis than their closely related relatives at Pennsylvania State University. Also, the only parthenogenetic embryos found during this 5-year study were in eggs at Beltsville. The first embryo appeared among the 780 eggs laid by the 16 original birds after their vaccination for fowl pox. Two embryos were found at Beltsville in 1963 among the 535 eggs laid by the 17 vaccinated hens and two embryos in 1964 among the 870 eggs laid by the 38 vaccinated turkeys.

In 1965, fertile eggs from vaccinated PG stock at Beltsville were transferred to Pennsylvania State University, where they were hatched, and the poults were raised in an environment free of fowl pox virus. Twenty-four virgin turkeys were raised to maturity and their eggs tested at the university in 1966. Five embryos were found among 986 eggs in the absence of fowl pox virus. Whatever the stimulus involved with embryo formation, clearly that stimulus was carried over in the hatching eggs and expressed itself at the university in progeny that had come from vaccinated birds at Beltsville and in the absence of further vaccination for fowl pox (87).

The results indicate that the live fowl pox virus has an active role in parthenogenetic development. Selection affected the average level of parthenogenesis (unorganized growth) in eggs of nonvaccinated PG turkeys at Pennsylvania State University; the increase through selection was from 1.1 percent in 1962 to 18.6 percent in 1966. The selection pressures, however, did not result in the production of hens capable of producing unfertilized eggs that would give rise to embryos.

Table 9.--Incidence of parthenogenetic development in unfertilized eggs of Pozo Gray turkeys at Agricultural Research Center (ARC), Beltsville, Md., and Pennsylvania State University (PSU), University Park.

Year and testing station	Line of turkeys 1/	Hens	Eggs	Eggs found to contain--			Parthenogenetic development
				Membrane only	Blood and membrane	Embryos	
		Number	Number	Percent	Percent	Percent	Percent
1962:							
ARC----------	ARC	16	780	3.9	0	0.1	4.0
PSU----------	PSU	16	474	1.1	0	0	1.1
1963:							
ARC----------	ARC-H	17	535	6.7	0	.4	7.1
PSU----------	PSU-H	13	153	5.2	0	0	5.2
PSU----------	PSU-L	14	167	1.2	0	0	1.2
1964:							
ARC----------	ARC-H	38	870	7.8	.5	.2	8.5
PSU----------	PSU-H	33	931	6.8	.2	0	7.0
PSU----------	PSU-L	37	1,217	1.1	0	0	1.1
1965:							
ARC----------	ARC-H	24	975	14.9	1.2	.5	16.6
PSU----------	PSU-H	37	1,234	8.4	.2	0	8.6
PSU----------	PSU-L	54	2,077	.4	0	0	.4
1966:							
ARC----------	ARC-H	24	1,022	19.1	1.6	.4	21.1
PSU----------	ARC-H	24	986	15.7	2.8	.5	19.1
ARC----------	PSU-H	22	866	18.6	1.2	.3	20.1
PSU----------	PSU-H	22	627	15.9	2.7	0	18.6
ARC----------	PSU-L	18	625	2.8	.1	0	2.9
PSU----------	PSU-L	18	593	1.0	0	0	1.0

1/ H = high incidence; L = low incidence.

These results support findings of Olsen (62, 71), who reported an increase in parthenogenesis in BSW turkey eggs from 16.7 to over 40 percent in 9 years. In the same period at Beltsville the incidence of embryos increased from 0.2 percent in 1954 to approximately 12 percent in 1965. However, BSW turkeys were selected for a high incidence of parthenogenesis in the presence of live fowl pox virus. Furthermore, young turkey females on test each year received a booster shot of virus near maturity as well as the original routine vaccination as young poults.

Test 5

The following tests for parthenogenesis include fertilized turkey eggs that had not been selected for a high incidence of parthenogenesis. Naturally occurring parthenogenesis at Beltsville can be expected to average each year about 16 to 20 percent (92, 93).

In 1960 Pennsylvania State University obtained hatching eggs from this unselected stock. Five generations of turkeys were reproduced at the university from stock originally imported as hatching eggs from Beltsville. Poults hatched at the university were not vaccinated for fowl pox.

In 1966, 22 of the young nonvaccinated virgin females from Pennsylvania State University were transferred to Beltsville, where they were kept isolated from other turkeys, and their unfertilized eggs were tested for parthenogenesis. Eleven other nonvaccinated birds, which were full sisters of those at Beltsville, were retained at the university, where their unfertilized eggs likewise were tested for parthenogenesis.

The 22 nonvaccinated virgin turkeys at Beltsville laid 746 unfertilized eggs, of which 33 or 4.4 percent underwent some degree of parthenogenetic development. The 11 nonvaccinated virgin turkeys retained at the university laid 311 unfertilized eggs, of which 15 or 4.8 percent underwent parthenogenetic development. Only one parthenogenetic embryo was found among the 746 unfertilized eggs at Beltsville; none were found among the 311 unfertilized eggs at Pennsylvania State University. These data show that on an average the level of parthenogenesis tends to decrease in eggs of virgin females that are several generations removed from fowl pox vaccination.

As a control, 984 additional unfertilized eggs laid by 20 unselected young virgin BSW turkeys were tested for parthenogenesis. These 20 females, like the aforementioned birds, were descendents of the original flock supplying hatching eggs for the university. Unlike the others, they were hatched and raised at Beltsville and had been vaccinated for fowl pox as young poults and again at sexual maturity. On an average, 18.3 percent of parthenogenesis was found among the 984 eggs. Among the 180 eggs undergoing parthenogenetic development were 17 embryos, which represent 1.7 percent of the total eggs incubated.

These data provide further evidence that the vaccination of unselected virgin BSW turkeys for fowl pox has an enhancing effect on the level of parthenogenetic embryos and on the overall incidence of parthenogenesis in

their unfertilized eggs. It should be noted that in 1952 the original unselected stock at Beltsville had an incidence of nearly 17-percent parthenogenetic development and that the same unselected stock reproduced at Beltsville without regard to parthenogenesis was still about 17 percent in 1966 under the same management conditions.

Twin, triplet, and quadruplet parthenogenetic embryos have been found frequently in unfertilized eggs laid by unselected BSW turkeys that had been recently vaccinated with live fowl pox virus. In one test 14 percent of all parthenogenetic embryos were twins (68). In one instance 8 embryos were found on a single blastoderm of a single-yolk unfertilized egg and in another instance 12 embryos were found. All these embryos were well formed and appeared to have reached approximately stage 13 (110, p. 132). Blastoderms of two unfertilized eggs, one on which parthenogenetic twin embryos have developed and one on which eight embryos have developed, are shown in figure 8.

Kosin and Sato (42) also reported finding a high level of twinning among parthenogenetic embryos (17.1 percent) in eggs of Broad Breasted Bronze turkeys. They did not state whether this high incidence of twinning was observed in eggs laid after fowl pox vaccination or after a natural outbreak of this viral disease.

Turkeys - Rous Sarcoma Virus

To test the effectiveness of live Rous sarcoma virus on parthenogenesis, 30 unselected BSW turkey females, housed in individual cages, were used. Of the hens 18 were vaccinated subcutaneously in the wing webs with 0.1 gram equivalent of partly purified Rous sarcoma virus. (One gram equivalent represents the quantity of virus extracted from 1 gram of tumor tissue.) Ray Bryan of the U.S. Public Health Service, Bethesda, Md., supplied this virus preparation and provided technical assistance in the vaccination of the turkeys.

The other 12 hens, full sisters of those of the treated group, served as unvaccinated controls. From both groups of females unfertilized eggs were incubated for 10 days before the eggs were broken and examined macroscopically for parthenogenetic development. The average incidence of parthenogenetic development was observed over successive 6-day intervals in eggs laid by treated and control birds. Table 10 shows the average levels of parthenogenesis observed in the unfertilized eggs at various intervals after vaccination. Only the unorganized type of development increased after vaccination with this ribonucleic acid (RNA) virus; that is, the incidence of parthenogenetic embryos did not increase.

Figure 8.--Above, twin parthenogenetic turkey embryos: Left, at 6 to 7 days; right, at 3 days. Below, 8 parthenogenetic turkey embryos on single blastoderm, each at approximately 3 days; two of them share same amniotic sac. (After Olsen and Poole (96).)

Table 10.--Incidence of parthenogenetic development in unfertilized eggs of Beltsville Small White turkeys, laid after vaccination of hens with live Rous sarcoma virus, Beltsville, Md.

Days after vaccination (number)	Vaccinated turkeys		Controls	
	Eggs	Parthenogenetic development	Eggs	Parthenogenetic development
	Number	Percent	Number	Percent
22 to 27------	72	11.1	53	15.1
28 to 33------	69	22.6	52	13.5
34 to 39------	59	20.3	42	16.7
40 to 45------	65	32.3	46	15.2
46 to 51------	65	26.1	50	16.0
52 to 57------	67	35.8	49	10.2
58 to 63------	64	28.6	46	15.2
64 to 69------	74	21.6	50	16.0
70 to 75------	53	15.1	46	26.1
76 to 81------	56	33.9	42	16.7
82 to 90------	70	28.6	51	15.7
Total or average	714	25.4	527	15.9

During the 90-day test after vaccination, 714 eggs were produced by the 18 vaccinated turkeys and 527 by the 12 control birds. Of all eggs produced by the treated birds, 25.4 percent developed parthenogenetically. Of the 527 control eggs, 15.9 percent developed parthenogenetically. The difference, when tested by the analysis of variance using weighted squares of means, was found to be highly significant at the 1-percent level. The results clearly indicate that live Rous sarcoma virus tends to increase the incidence of macroscopically observable parthenogenetic development (unorganized) in unfertilized turkey eggs (65).

Turkeys - Newcastle Disease Virus

The effect of Newcastle disease virus (NDV) on parthenogenesis was conducted by Eugene Gill, Food and Drug Administration, and Henry Stone, National Animal Disease Laboratory, Ames, Iowa. NDV virus was chosen because it produces viremia on entering the blood circulation and can be easily isolated during the first 72 to 96 hours after vaccination or challenge (Gill and Stone, pers. commu.). Initiated in 1960, tests involved 42 unselected BSW turkey females, approximately 36 weeks of age. In the treated group were 21 birds vaccinated on January 6, 1960; the other 21 hens were the controls.

Eggs collected daily from both groups were placed each evening in an incubator which operated at 99° F and at 62 percent relative humidity. After an initial 10-day incubation period, the eggs were removed, broken, and examined macroscopically for parthenogenetic development. The turkey hens

came into egg production at the end of the third week after this live virus vaccination. The average percentage of parthenogenetic development found at weekly intervals in eggs of both treated and controls groups of turkeys is shown in table 11.

Table 11.--Incidence of parthenogenetic development in unfertilized eggs of turkeys, laid after vaccination of hens with live Newcastle disease virus and revaccinated 11 weeks later, Beltsville, Md.

Weeks after vaccination (number)	Vaccinated turkeys		Controls	
	Eggs	Parthenogenetic development	Eggs	Parthenogenetic development
	Number	Percent	Number	Percent
4----------	35	54.0	39	10.4
5----------	51	33.0	61	16.4
6----------	51	23.5	63	12.7
7----------	62	17.7	56	16.1
8----------	87	20.5	56	16.1
9----------	87	16.0	55	12.7
10----------	91	14.0	53	17.0
11----------	50	32.0	50	10.5
12----------	39	12.8	50	24.0
13----------	48	14.5	53	14.8

Source: Gill, E., and Stone, H. (Unpub. data).

NDV brought about a marked increase in the percentage level of parthenogenetic development in unfertilized eggs of the treated group. Differences in percentage levels of parthenogenetic development in unfertilized eggs of the treated group and controls were highly significant when tested by the analysis of variance.

EFFECT OF KILLED VIRUS VACCINES ON PARTHENOGENESIS IN TURKEYS

The tendency of live virus vaccines, such as those of fowl pox, Rous sarcoma, and Newcastle disease, to induce parthenogenesis in turkey eggs raised the question whether the same response might be elucidated by using killed virus vaccines.

In this study 80 virgin BSW turkey hens, approximately 36 weeks old, were unselected as to parthenogenesis. All birds maintained in laying cages and on a 14-hour light day received the same all-mash turkey laying diet. They were assigned at random to 4 groups composed of 20 birds each. Birds of group 1 were given subcutaneous injections of killed NDV; those of group 2, killed fowl pox virus, and those of group 3 killed RSV. All viruses were inactivated by 0.2 percent solution of beta-propriolactone. The 20 birds of group 4 served

as controls; 12 were inoculated with 0.5 ml of a suspension of macerated embryonic tissue of 12-day chicken embryos; and 8 received no injection.

Unfertilized eggs were collected daily and identified by hen number and date of lay. Each evening, daily collections of eggs were placed in an incubator at a temperature of 99.7° F and a relative humidity of 57 percent. All eggs were incubated 10 days before candling. At time of candling eggs containing viable embryos were returned to the incubator. Unfertilized eggs and dead embryos were removed and subsequently opened for a more critical inspection of the germinal disk for parthenogenetic development. The incidence of parthenogenesis was recorded for 25 days before vaccination with inactivated virus and for 60 days after vaccination. Results of each treatment are presented in table 12 (67).

Table 12.--Incidence of parthenogenesis in eggs of Beltsville Small White turkeys laid before and after vaccination of the hens with inactivated Newcastle disease, fowl pox, and Rous sarcoma viruses, Beltsville, Md.

Treatment	Group No.	Before vaccination		After vaccination	
		Eggs	Parthenogenetic development	Eggs	Parthenogenetic development
		Number	Percent	Number	Percent
Turkeys vaccinated with:					
Newcastle disease-----	1	266	19.8	501	19.0
Fowl pox virus--------	2	272	16.2	697	20.7
Rous sarcoma virus----	3	218	19.3	507	19.1
Controls:					
Macerated chicken embryo----------	4	153	21.6	368	17.9
No treatment----------	4	99	17.0	213	18.8

Data showed that none of the killed virus preparations used to vaccinate turkey hens had any detectable effect on the level of parthenogenesis in their eggs. These tests rule out the possibility of components present in live virus vaccines, such as tissue cells and blood, as causative agents. The same materials were present in the killed virus preparations, which proved to be ineffective in inducing parthenogenesis, and in the macerated embryonic tissues used to inoculate 12 of the 20 controls. Antibodies can also be discounted as causative agents for parthenogenesis because NDV, although inactivated, stimulates the production of antibodies. Furthermore, commercial vaccines containing inactivated NDV are commonly used by poultrymen to protect chickens against infection caused by this RNA virus.

The lack of response of turkey hens to the three viral preparations inactivated with beta-propriolactone indicates that the causative agents are active viruses. The killed virus particles, when injected subcutaneously, either fail to reach the ovary or are unable to penetrate the vitelline membrane of turkey germ cells.

Just how live fowl pox, Newcastle disease, Rous sarcoma, and leukosis viruses react with certain chicken and turkey germ cells, enabling them to undergo parthenogenetic development, is unknown. The action of the desoxyribonucleic acid (DNA) fowl pox on germ cells may be somewhat different than that of the three RNA viruses. After use of live fowl pox virus, the relative numbers of embryos, including twins increases significantly (127). However, use of the RNA viruses resulted in an increased incidence of only an unorganized type of development (65, 73). This suggests that fowl pox virus may be serving both as an organizer and a stimulant to cellular proliferation.

When found, both DNA and RNA viruses seem to persist and the acquired predisposition to parthenogenesis is transmitted to the progeny of inoculated turkeys even though their progeny are not revaccinated (73, 87). This prolonged effect suggests that the genotype of certain birds may have been altered in some manner, enabling future generations to produce unfertilized eggs with a strong predisposition toward parthenogenesis. However, only certain hens are so affected by the virus. In the case of the natural outbreak of leukosis in WL chickens, only 4 of 17 hens acquired the predisposition toward parthenogenesis (73).

Olsen (61, 67, 73), Olsen and Poole (96), and Olsen and Buss (87), contended that certain microorganisms are associated with parthenogenetic development in unfertilized eggs of genetically susceptible chickens and turkeys. Stolk (125) found three cases of parthenogenesis in viviparous toothcarps in which the stimulus required for the start of parthenogenetic development in unfertilized oocytes consisted in a localized infection with the Ichthyophonus parasite, Phycomycete Ichthyophonus hoferi, Plehn-Mulsow.

Stolk (126) established a relationship of the Ichythoyophonus parasite and the development of two teratomas on the ovaries of fish. One ovarian teratoma was found among 1,484 adult females of the viviparous fish Lebistes reticulatrus examined and another teratoma among 1,179 adult virgin females of Xiphophorus maculatus. No teratomas, however, were found among 946 adult virgin females of X. helleri. The coincidence of the Ichthyophonus infection and the ovarian tumors led Stolk (126) to conclude that development of the two ovarian tumors was caused by the Ichthyophonus toxin, which provided also the stimulus for parthenogenetic development.

Stolk stated, "from this point of view pathological parthenogenesis may be regarded as a form of artificial parthenogenesis, effected not so much by experimental intervention as by nature itself." He also stated (126), "In our opinion it is undoubtedly possible that the cases of parthenogenesis in Lebistes described by Spurway might also involve a pathological process, probably an Ichthyophonus infection which is by no means uncommon in fish; indeed its incidence is very high, particularly in the viviparous toothcarps as it is highly infectious."

Both Loeb (46) and Lusted (47) recognized a possible relationship between parthenogenesis and some types of ovarian tumors.

Leroy Stevens (pers. commun.) of the Jackson Laboratory, Bar Harbor, Maine, found a tumorgenic inbred strain of mice. Of the virgin females, 3 percent produced some eggs which had undergone a spontaneous form of parthenogenesis. The embryos developed quite normally for 6 or 7 days, but then they died.

Spurway (121) found instances of parthenogenesis among L. reticultus. Later (122) she sectioned ovaries of some fish and found evidence of testicular tissue and sperm. Understandably, the earlier interpretation of parthenogenesis was changed to hermaphroditism with self fertilization. Stolk (125) and Olsen believed that the hermaphroditism observed by Spurway (122) in Lebistes could have been acquired and could have resulted from some infection of the ovary. Because of some pathological condition of the ovary, adult domestic chicken females commonly have taken on the plumage, appearance, and even some of the activities of the male. For example, Gatenby and Brambell (30) described an adult White Leghorn female that, in the course of time, came to resemble a cock bird. The bird courted other hens and crowed often and loudly. An ovary full of scar tissue and containing testicular tubules was on its left side.

Hermaphroditism was likewise checked and eliminated as a possible cause of fertility in eggs of virgin birds. Ovaries of virgin turkeys of various ages were removed, sectioned, and subsequently examined for the presence of testicular tissue. All results were negative.

The spontaneous parthenogenesis described in chicken and turkey eggs does not stem from a pathological condition of the ovary, in the sense that visible lesions are present. Almost every turkey hen in the flock, at some time during the season, will produce some unfertilized eggs that upon being incubated will develop parthenogenetically to some degree. Furthermore, if blastodisks of unfertilized BSW turkeys eggs are sectioned, almost all of them will contain nucleated cells. Hens producing these eggs are perfectly healthy and each lays the expected number of eggs. Some hens have been retained for more than one season and during their second and third laying year, they still produce eggs with a high level of parthenogenetic development (78).

The exact way in which the live virus enhances parthenogenetic development in unfertilized eggs of susceptible chickens and turkeys has not been determined. However, the virus on gaining entrance into ova may possibly be attaching to the genetic material. If this proves correct, then viral DNA could be altering meitotic cell divisions in such a way that ova are capable of undergoing parthenogenetic development.

Dobzhansky (28, p. 214) stated, "Meiosis, like any other physiological process, is ultimately controlled by the genotype of the organism. The normal course of meiosis involves a definite succession of events which are so delicately adjusted that a failure of any one of them, or simply a change in the normal time relationships, may cause a dearrangement of the whole process. Gene mutations can attack this process at any stage. Consequently, chromosome conjugation may fail despite the presence of pairs of chromosomes having similar genes arranged in identical lineal series."

Baltimore (6) stated, "When a virus invades a cell, it establishes in it a new genetic system that allows for the replication of the viral genetic material and the expression of the information encoded in the viral genome. The genetic system may allow for the stable integration of the viral genetic materials into the cell.... The genetic systems of DNA viruses are basically miniature replicas of the host's own system: the information is encoded in DNA that can replicate itself (or be replicated) and is transcribed into messenger RNA (mRNA) molecules that can be translated into protein. The replicative system of DNA viruses probably have elements not found in the host cell systems, but, in outline, the host and viral genetic systems are quite similar."

Stoker (124) exposed hamster cells to large doses of the DNA polyoma virus. A small proportion of the cells were transformed permanently. A much higher proportion of infected cells were transformed temporarily when at least one characteristic of transformation was acquired for a few (up to six) cell divisions and then lost again. Acquisition of the transformed phenotypes, therefore, need not be permanent and does not depend upon stable integration of the viral genome.

Rhim and others (108) reported a spontaneous transformation of rat embryonic cells after their long term in vitro cultivation. These cells maintained in tissue culture for long periods developed into tumorgenic lines that exhibited morphological alteration. The authors stated, "The present study describes the first demonstration of in vitro spontaneous transformation of rat cells. The following criteria established that the in vitro transformation had occurred in the RE cell lines described: (a) The cells were morphologically altered and grew as randomly oriented multilayers. (b) The cells showed increased growth rate and lost contact inhibition. (c) Inoculation of the altered cells into unconditioned rats produced malignant, progressive, transplantable tumors."

NORMAL CYTOLOGY OF FEMALE MEIOSIS

Maturation Changes

The nucleus or germinal vesicle in the smallest follicles of the adult chicken ovary is located near the center of the developing oocyte. At this early stage of growth, the nucleus is relatively large compared with the total volume of the cytoplasm of the follicle. The chromatin material in these small follicles stains readily with such basic stains as Delafield's hematoxylin and with Heidenheim's iron hematoxylin. As the young ova increase in size and enter into what is termed "the rapid growth phase," the nucleus moves toward the periphery of the cell and eventually comes to lie (45) in the center of a thickened mass of protoplasm known as the germinal disk.

In mature ovarian follicles, the outer surface of the germinal vesicle is flattened against the vitelline membrane while its inner surface remains convex. The germinal vesicle is surrounded by a thin but definite nuclear membrane. The interior structure of this vesicle appears to be composed of a fine network of delicate fibers and within this network is a clear, nuclear

sap. Near the time of ovulation, the germinal vesicle has lost much of its affinity for Delafield's and Heidenheim's hematoxylin stains. Within the meshwork of the germinal vesicle of mature follicles are scattered strands of chromatin which can be seen in certain well-stained sections. Near the time of ovulation, the chromatin is less scattered and is found near the center of the vesicle.

In both chicken and turkey ova, maturation changes, as indicated by the disintegration and subsequent disappearance of the large germinal vesicle, become apparent approximately 8 to 10 hours before ovulation (91). These changes occur in all chicken and turkey eggs whether eggs are destined to be fertilized or to develop parthenogenetically.

The first polar body is extruded a few hours before ovulation. The first stages in the formation of the second polar body also occur before the ovum is released from the follicle. However, the process is not completed until after ovulation and in mated birds after entrance of the sperm (59, 90). Figure 9 shows the nuclear changes that occur just before and immediately after ovulation.

All the preovulatory nuclear changes are initiated by or at least are associated with the release of the luteinizing hormone of the fowl's pituitary gland. These changes have been induced experimentally in chickens after appropriate injections of the ovulating-inducing hormone (91). Thus both physiological and genetic influences are involved in the initiation of nuclear changes which have been in operation for a number of hours before ovulation.

Determination of Sex in Birds

The mode of sex determination in birds is the reverse of that found in mammals and in most other animals. In birds, the female is the heterogametic sex, that is, she produces two types of germ cells. One type of ovum carries an X-sex chromosome; the other carries a Y-sex chromosome. The male fowl produces only one type of spermatozoa; that is, each spermatozoon carries a single X-sex chromosome. At fertilization, when an X-bearing sperm fuses with an X-bearing ovum, the resulting zygote will give rise to a male; when an X-bearing sperm and a Y-bearing ovum unite, the resulting zygote will give rise to a female. In mammals, a mammalian zygote having two-like sex chromosomes (XX) will give rise to a female; one having the XY combination, to a male.

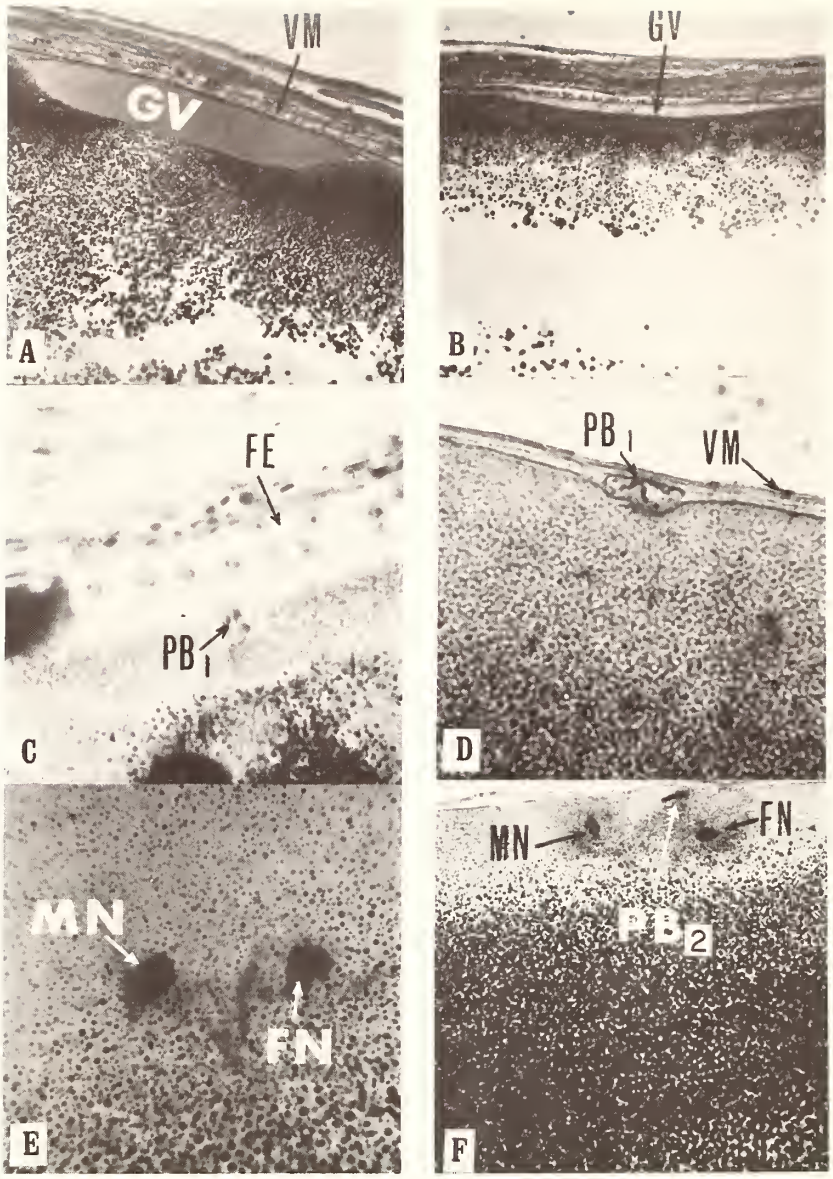

Figure 9.--Nuclear changes occurring in ova of chickens and turkeys just before and immediately after ovulation: A, Median cross section of germinal disk of a turkey ovarian follicle removed from the ovary 24 hours before time of ovulation showing the large germinal vesicle (GV) just beneath the vitelline membrane (VM). (X 150.) B, Cross section of the germinal disk of a chicken ovarian follicle obtained just before ovulation showing GV spreading laterally as a thin sheet beneath the VM. (X 75.) C, Cross section of the blastodisk of a mature chicken ovarian follicle showing the first polar body (PB_1) at anaphase and cells composing the follicle epithelium (FE). (X 720.) D, Cross section of the blastodisk of a newly ovulated ovum of a chicken showing a first polar body (PB_1). (X 720.) E, A median cross section of the germinal disk of a recently ovulated turkey ovum from the infundibulum showing male (MN) and female (FN) pronuclei. (X 720.) F, Cross section of the blastodisk of a newly ovulated chicken ovum showing the male (MN) and female (FN) pronuclei near the second polar body (PB_2). (X 360.)

Cells of parthenogenetic origin in chicken and turkey eggs generally contain the diploid number of chromosomes (41, 101, 136). This raises the question as to the cytological route by which diploidy is restored. Beatty (11, 12) listed several routes that a germ cell may follow in acquiring a diploid set of chromosomes. Briefly these are: (1) The suppression or reentry of the first polar body. (2) Suppression or reentry of the second polar body. (3) A nuclear division at mitosis I without a corresponding cytoplasmic division. (4) Fusion of two haploid mitotic products, sometime after mitosis I.

A nuclear division in the absence of a cytoplasmic division (route 3) in a haploid cell or a fusion of two haploid mitotic cells sometime after mitosis I (route 4) could lead to diploidy. Parthenogens arising from an egg following either of these two routes should be completely homozygous at every locus. Such is not the case, however, with all parthenogenetic turkeys, because some parthenogens can be heterozygous at certain loci (74, 103, 104). Routes 3 and 4 do not appear to be the possible modes of restoration of diploidy in these cases.

Sex inheritance in birds as noted earlier is the reverse of that found in mammals. The germ cells of the male turkey carry two XX sex chromosomes, and the germ cells of the heterogametic female carry the XY pair. Suppression or reentry of the first polar body should, theoretically, give rise to some female parthenogens. However, all sexually mature parthenogens thus far found have been males, with possibly two exceptions. 6/ Further, the inspection of gonads of 67 fully developed parthenogenetic embryos revealed that all were males (105). Sato and Kosin (115) also established that cytologically cells present in developing membranes carry the two XX sex chromosomes. They concluded that the genetic sex of the membrane growths and of embryos was male. Because no female parthenogens have been reported, very likely the first polar body (route 1) is not involved in the restoration of diploidy.

Suppression or reentry of the second polar body is another route by which diploidy could be restored. This route to diploidy would produce parthenogens that could show heterozygosity to some degree. Preceded by normal meiotic reduction and crossing over, this route would produce the autosomal diploid equivalent of a fertilized egg containing chromosomes heterozygous within crossover regions for any loci at which the dam was also heterozygous.

In eggs of birds neither the first nor the second polar bodies are actually extruded. Polar bodies always remain in shallow depressions in the protoplasm at the upper surface and near the center of the germinal disk,

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6/ Two adult individuals were encountered in the course of this 20-year study which phenotypically were classified as females. One of these turkeys accepted permanently a skin graft from her dam. At autopsy, a complete but infantile oviduct was found but no recognizable ovary. The second individual laid about 10 eggs before developing an ovarian tumor. The dam of this second hen was not available for a skin grafting test.

just beneath the vitelline membrane (59, 90, 91). They are, therefore, always in close proximity to the female pronucleus.

CYTOLOGICAL STUDIES

Several cytological studies have been conducted to determine ploidy of cells in embryonic tissues from unfertilized turkey eggs. Yao and Olsen (136) and Sarvella (111, 113) made chromosome counts of cells of embryonic membranes from eggs of BSW turkeys. Sato and Kosin (114, 115) used embryonic tissues from eggs of Washington State Whites and Broad Breasted Bronze turkeys. Cells having haploid, diploid, and tetraploid numbers of chromosomes were found. Poole (101) examined cells of pinfeathers of adult BSW parthenogens and established that these males were diploid. Stephen Bloom, Cornell University, (pers. commun.) recently confirmed this finding.

Darcey and Buss (25) and Darcey and others (26) determined the ploidy of cells of 16 blastoderms of newly laid BSW turkey eggs at 5 to 7 hours of incubation. Haploid and diploid cells, in about equal numbers, were encountered in these unfertilized eggs.

Sato and Kosin (114, 115), Darcey and Buss (25), and Darcey and others (26) postulated that the ovum starts mitotic division as a haploid cell. After a few divisions, some, but not all, mitotic cells fuse, or else blocked mitoses occur in certain haploid mitotic cells, thus restoring the diploid number of chromosomes. Diploid mitotic cells in subsequent divisions would then divide normally producing only daughter cells having the diploid number of chromosomes.

Blood Antigen Approach

If an animal is truly parthenogenetic, then it should have received a sample complement but not all of the genes possessed by the heterozygous dam. Such an individual should possess no antigens that are not already present in the dam.

Blood samples from parthenogens and their dams were submitted to Jack Law, of Hy-Line Poultry Farms, Des Moines, Iowa, for testing for antigens. Dr. Law found no evidence for the presence of hemaglutinogens in the blood of the parthenogen that were not already present in blood of their virgin dams (Law, pers. commun.). This finding indicates that no male was involved in the production of these particular birds.

Skin-Grafting Tests

In 1961 scientists at the Department of Surgery, College of Physicians and Surgeons, Columbia University, N. Y., cooperated in studies involving skin grafting designed to test known principles of histocompatibility and to test for heterozygosity in the parthenogen.

Fourteen turkey parthenogens and their dams were involved. Reciprocal skin exchanges were made between the 14 turkey parthenogens and their respective dams. All 14 grafts, including second sets from parthenogenetic males to their dams, were accepted. All grafts from dams to their parthenogenetic sons were rejected (37). The results obtained were to be expected if the males were truly of parthenogenetic origin.

A parthenogen receives only a sample of the genes possessed by his heterozygous dam so he lacks some of her antigens. When he received skin from his dam, his body recognized foreign material and rejected it. The dam has all the antigens present in the genotype of her parthenogenetic son, plus some additional ones. Therefore, the dam accepted skin from her parthenogenetic son permanently. In the course of skin exchanges, two full brother parthenogens and their dam were available. Reciprocal skin exchanges between full brother parthenogens were rejected, although each of the parthenogenetic brothers was able to donate skin successfully to his dam.

Further skin grafting studies were made between parthenogens and their offspring to determine if parthenogens were homozygous. If the parthenogen were completely homozygous at every locus, then each of his sons and daughters should have received a full complement of his genes and, therefore, should possess all antigens of their parthenogenetic sire.

Three parthenogenetic males were mated to unrelated BSW hens to produce sons and daughters. Skin exchanges were subsequently made between parthenogenetic males and their offspring by unrelated hens. All six offspring of one parthenogenetic male rejected their sire's skin. Of seven offspring of another parthenogenetic sire, six rejected and one accepted their sire's skin. One poult, a female was sired by the third male; she accepted her sire's skin. Thus, among 14 skin exchanges, between parthenogenetic males and their progeny by unrelated hens, only two accepted their sire's skin (104).

Two parthenogenetic males were backcrossed to their own dams to produce sons and daughters. Skin exchanges were subsequently made between each parthenogenetic sire and his offspring by his own dam. Wattle skin from one male was accepted permanently by three of his progeny and rejected by four. Skin from the second parthenogenetic male was accepted by two and rejected by two others of his progeny. On the basis of skin-grafting results Poole and others (104) and Poole (103) concluded that parthenogens can be heterozygous at at least one of the segregating loci assumed to control histocompatibility in turkeys.

Genetic Color Marker

To obtain parthenogenetic males of the proper genotype for tests on the genotype of parthenogens having bronze-colored plumage, BSW hens were first mated to homozygous bronze males. All virgin females from this cross had bronze plumage although they were heterozygous (Cc) at the locus controlling plumage color. Virgin heterozygous bronze females produced unfertilized eggs from which several parthenogenetic males having bronze plumage hatched.

If a given parthenogen were homozygous (CC) at the C locus on an autosome controlling color, all poults sired by him, upon being mated to BSW females, would carry one dominant gene for color. His poults, therefore, would have bronze plumage.

Four parthenogenetic males each with bronze plumage were mated with virgin BSW hens. Down color of embryos and poults were recorded. Three of the parthenogenetic males sired bronze poults, which indicated that each of these parthenogenetic sires were homozygous (CC) at the locus controlling plumage color. The fourth bronze parthenogenetic male sired 137 poults of which 59 were white and 78 bronze, which indicated that this particular male was heterozygous (Cc) at the locus controlling plumage color (74 and Olsen, unpub. data). Thus, both skin grafting and color marker tests showed some parthenogens to be heterozygous at certain loci.

Olsen (74) and Olsen and Buss (88) found that nonmated heterozygous bronze turkey females (Cc) produced an equal number of white and colored parthenogenetic embryos and poults. This evidence suggests that homozygosity is occurring at this locus in the parthenogens produced. However, since the (C) locus for plumage color is close by the centromere, little, if any, crossing over may take place. In cases of heterozygosity, diploidy most likely is being restored through nondisjunction or refusion of the second polar body. The initial stages in the formation of the second polar body occurs while the ovum is still attached to the ovary. Formation of the second polar body is at metaphase when ovulation occurs and remains at this stage until, in the case of mated birds, entry of a sperm provides the necessary stimulus for its final completion (59, 76, 84). 7/

Because no sperm are present in parthenogenesis, the chromosomes of the egg nucleus and those of the second polar body probably never completely separate (11, 12, 109, 133). The unfertilized turkey ovum therefore starts division with the diploid number of chromosomes. Thus, we have in the turkey an automatic, faculative, arrhenotoky form of parthenogenesis; that is, either eggs can develop parthenogenetically producing only males or alternatively or they can be fertilized, in which case both males and females are produced.

Restoration of diploidy because of retention of the second polar body means that turkey parthenogens can be heterozygous when crossover occurs. The existence of heterozygosity in parthenogens was noted earlier, having been demonstrated through skin grafting and with the use of genetic color markers (74, 103, 104).

Restoration of diploidy at meiosis II also explains why fertility is attainable when hens, whose unfertilized eggs have exhibited a high incidence of parthenogenesis, are mated (26). When viable sperm are present in the infundibulum, meiosis II is completed. This allows for normal fusion of haploid male and female pronuclei. When viable sperm are absent, chromosomes of the second polar body and those of the egg nucleus may never separate completely. Subsequently, the egg nucleus may develop as a diploid cell.

7/ See footnote 4, page 6

One route to diploidy is through suppression or refusing of the second polar body; however, the possibility is fully recognized that an ovum may occasionally take an alternate route. As one example the fly, Drosophila mercatorum, might be cited. With the aid of appropriate genetic markers, Carson, Wei, and Neiderhorn (20) showed that 94 percent of the parthenogenetic flies are completely homozygous and are assumed to arise as a result of the fusion of two mitotic products. On the other hand, 6 percent of the flies are heterozygous and probably arise as a result of fusion of two meiotic products.

Bergerard (14) found that diploidy, in unfertilized eggs of Plasmidae (stick insect) was restored after mitosis I. Diploidy was restored after blockage of mitoses in haploid, mitotic cells. This route to diploidy should make parthenogenetic Plasmidae completely homozygous at every loci. Parmenter (99) also found that diploidy in parthenogenetic frogs may arise by more than one mechanism. He felt, however, that regulation to diploidy in tadpoles which approach or accomplish metamorphosis takes place before the first cleavage.

SOME CHARACTERISTICS OF TURKEY PARTHENOGENS

Developing Embryos

Parthenogenetic development in general is characterized by a 2- to 3-day delay in the onset of its development once the unfertilized eggs have been placed in the incubator. However, once parthenogenetic development is under way, growth of the developing embryo occurs superficially at a near normal rate. Initial delay followed by the near normal rate of development means that BSW parthenogenetic embryos usually require at least 29 days of incubation before they are ready to emerge from the shell.

Weights and body lengths of normal and parthenogenetic embryos of various ages were compared. Parthenogenetic embryos were derived from unfertilized eggs of turkeys that had been intensively selected for several years for an increased incidence of parthenogenetic development.

Normal embryos serving as controls came from fertilized eggs of a group of young BSW hens from a strain in which no attempt had been made to intensify the parthenogenetic trait. Each day, beginning on the 9th day and extending to the 25th day of incubation, three living parthenogenetic embryos and two normal embryos were sacrificed. When three parthenogenetic embryos were not available, then only two were weighed or measured. Each embryo minus its yolk sac was weighed to the nearest 0.1 gram. The length (millimeters) of each embryo was determined by measuring from the top of the head to the base of the tail. On each day of incubation the normal turkey embryos averaged heavier and longer.

Differences found between normal and parthenogenetic embryos, both in body weight and body lengths, are shown in figures 10 and 11. Differences show a delay of approximately 3 days throughout the embryonic period. The lower weights and lengths of parthenogenetic embryos can be attributed

largely to the characteristic delay on the part of the blastoderms of unfertilized eggs in resuming development once the eggs had been placed in the incubator (70, 79).

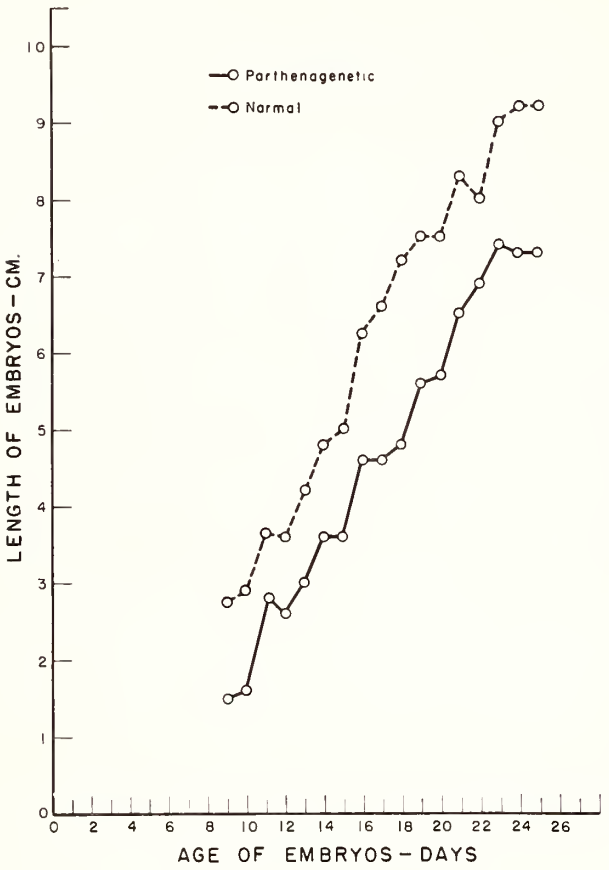

Figure 10.--Average weights of three parthenogenetic and two normal Beltsville Small White turkey embryos on given days of incubation.

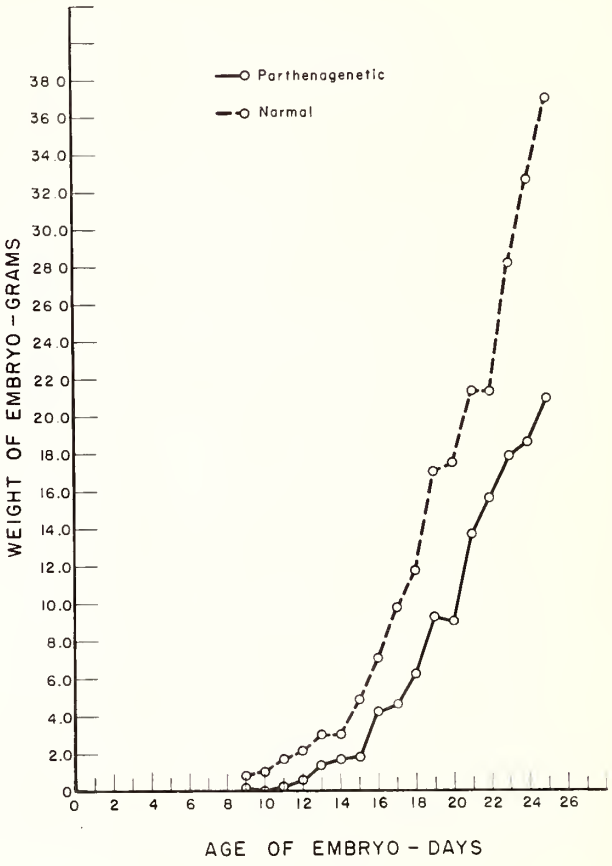

Figure 11.--Average body lengths of three parthenogenetic and two normal Beltsville Small White turkey embryos on given days of incubation.

Average weights were determined for some internal organs and glands of 21 fully developed parthenogenetic embryos and compared with those of 21 normal turkey embryos. Average weights of embryonic testes, spleens, thyroids, hearts, and livers were determined. All parthenogenetic embryos had reached a stage of development at which the beak normally would have been expected to penetrate the air cell, but none had hatched. Beltsville Small White (BSW) embryos from the same high incidence strain but obtained from fertilized eggs were the controls. The live, normal control embryos were removed from the incubator 48 hours before the expected time of hatching in order to compensate, at least in part, for the time lag in onset of development exhibited by the parthenogenetic turkey embryos (table 13).

Table 13.-- Least Square Means: Average weights of organs and glands of fully developed normal and of parthenogenetic embryos (adjusting for embryo weights), Beltsville, Md.

Organ or gland	Weight in-- Normal embryos	Parthenogenetic embryos
	Mg	Mg
Testes	8.58*	2.47
Spleen	15.61**	12.55
Thyroid	6.65*	4.40
Heart	234.79	257.23
Liver	830.46*	965.96

* = means differ significantly at 1-percent level of probability. ** = means differ significantly at 5-percent level of probability.

Average weights of testes, spleens, and thyroids of parthenogenetic embryos were significantly lower than those of normal embryos. The greatest variability was in testes weights; those from parthenogenetic embryos averaged only 28.8 percent of the adjusted weight of the controls. Testes weight among individual parthenogenetic embryos varied from 0.9 to 8.7 mg, a nearly tenfold difference, while among embryos from fertilized eggs, the difference was 5.0 to 11.0 mg, a 2.2-fold difference (81).

Newly Hatched Parthenogens

Parthenogenetic turkey poults at time of hatch are less vigorous and much weaker than those from fertilized eggs. Slow in hatching many of them cannot emerge from the shell without assistance. To survive most parthenogenetic poults must be given special care and provided with special brooding facilities.

In learning to eat and drink, young turkey poults generally are slower than chicks. Weak and highly inbred, parthenogenetic poults require even more care than normal poults. At Beltsville, we hand-raised each individual parthenogen. Even with this special care, only about 20 percent of the parthenogenetic poults that hatch survived to maturity.

Young parthenogenetic poults were given their first food through a rubber catheter threaded down their throats and into their crops. The catheter was attached to a hypodermic syringe. Each young parthenogen received about 1/4 ml of a liquid "egg-milk-vitamin" mixture, twice daily, for the first few days. In time, poults grew stronger and learned to eat. Figure 12 shows a young parthenogen in the process of being fed and taught to eat.

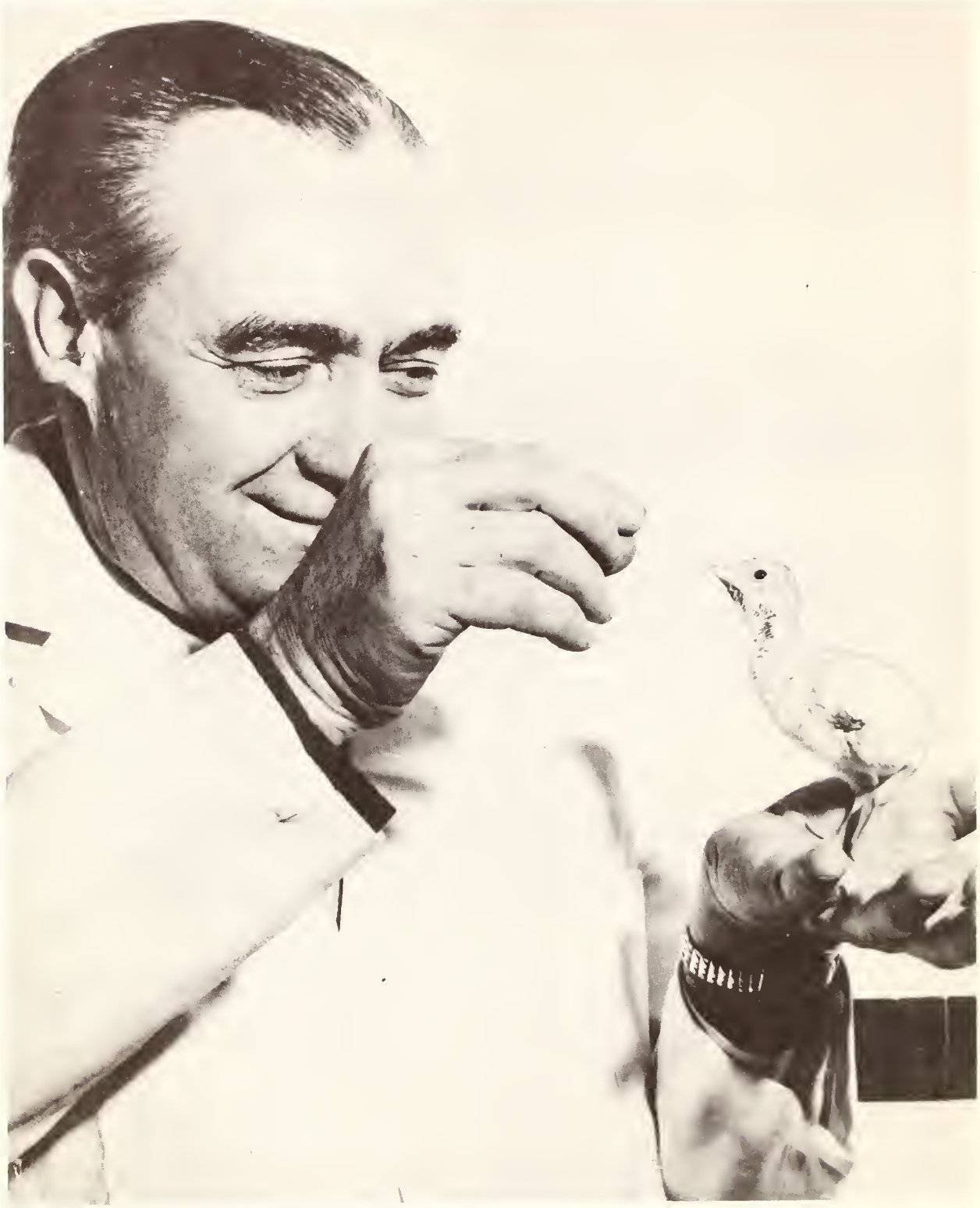

Figure 12.--Young parthenogen being fed and taught to eat.

Brooding also presented a problem because many of the newly hatched parthenogens could not stand or tended to fall often. When the poults fell, they had considerable difficulty in regaining their balance. In a conventional brooder, young parthenogens often fell in areas where temperatures were either too high or too low. This resulted in heavy mortality. A small glass-covered incubator that served as brooder overcame this problem. The incubator provided an environment in which both light and temperature in all areas of the brooder could be controlled. Furthermore, the poults were kept confined to a small area and were always close to feed and water. E. G. Buss, Pennsylvania State University, observed that newly hatched chicks as companions aided parthenogens to eat and drink. Young poults were kept in this controlled environment until they learned to eat, drink, and become stronger. Subsequently, they were transferred to more conventional types of brooders.

Adult Parthenogens

The testes of mature parthenogenetic males is the organ most often nonfunctional. Only one in five parthenogenetic males at maturity can be expected to produce semen (71, 80, 81). Although the quantity of semen produced by most parthenogenetic males tends to be limited, its quality is little different from that produced by normal males. Levels of fertility and hatchability obtained from eggs of 14 BSW hens after insemination with semen from one parthenogenetic male are shown in table 14 (62).

Failure of 80 percent of adult parthenogens to produce semen possibly may be traced to the general retarded condition of their reproductive organs at time of hatching. Failure of their testes and possibly certain other endocrine organs to develop normally may be reflected indirectly in the abnormal behavior of parthenogens.

Olsen (86) determined weights of 5 internal organs and glands of 12 adult (18 months old) parthenogenetic BSW males. Weights of corresponding organs and glands of 12 adult (13 months old) normal BSW males were likewise obtained. The testes of most adult parthenogens were underdeveloped. The average weight of the testes of the 12 unlighted adult parthenogens was only 2.4 g. This compares with an average weight of 23.5 g for the testes of normal unlighted males. This marked difference in weights explains why only 20 percent of the parthenogens on the average produces semen at maturity.

Livers of adult parthenogenetic males on the average were significantly heavier than those of normal males. Average weights of spleens, hearts, and thyroids were nearly the same as weights of the same organs in normal males.

Fifty-five parthenogens hatched between 1955 and 1971 produced limited quantities of semen. Semen collected from 26 of these males was used to inseminate young virgin and older, nonmated BSW hens. Each of these 26 males sired several normal, healthy poults (70 and unpub. data). One of the first parthenogenetic males to produce semen, along with one of the first poults sired by him is shown in figure 13 (4, 16, 24, 47, 117).

Table 14.--Incubation record of eggs produced by 7 virgin and 7 previously mated Beltsville Small White turkeys after those hens had been inseminated with semen from one parthenogenetic male, Beltsville, Md.

Type hen	Eggs laid after insemination	Fertile eggs 1/	Dead embryos after--		Poults hatched
			1 to 14 days	15 to 28 days	
	Number	Percent	Percent	Percent	Percent
Virgin hens----------	189	50.8	8.3	6.3	83.3
Previously mated hens-	131	38.9	13.7	3.9	82.4

1/ Percentages based on fertile eggs, except those in column 4 which are based on total eggs.

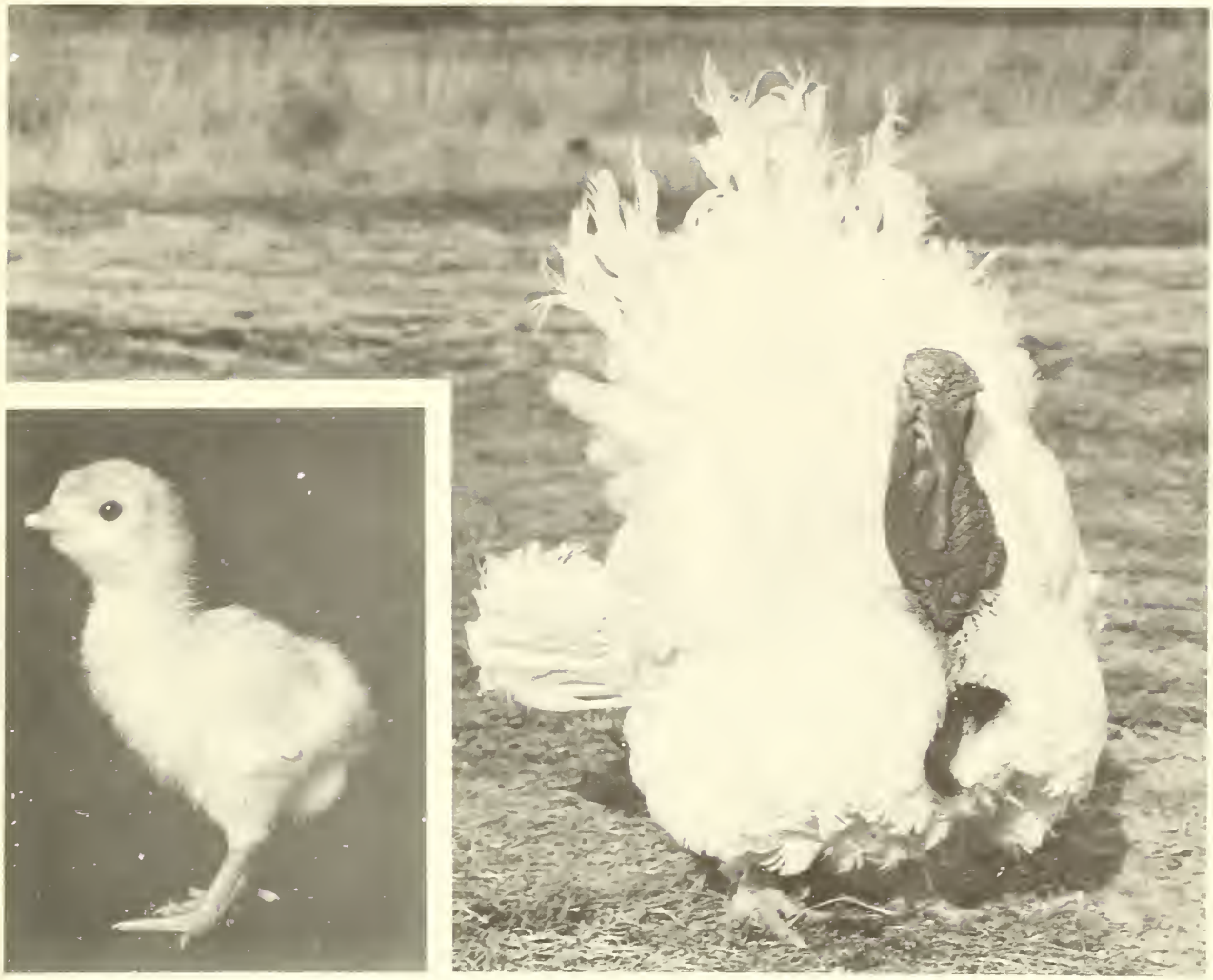

Figure 13.--Parthenogenetic male No. 1 and (inset) newly hatched normal poult sired by him.

Longevity

Olsen (86) conducted a study on the longevity of BSW parthenogenetic turkey males. Records of 50 mature parthenogens were reviewed in determining the lifespan of these unique birds. The 50 parthenogenetic males involved were hatched between 1958 and 1967. As young adults the parthenogens were transferred to small 12 foot x 12 foot unheated colony houses where they remained confined until death. The parthenogens were maintained in floor pens on straw litter and kept segregated from normal turkeys.

Of the parthenogens 46 (92 percent) died between November 1958 and November 1971. Parthenogenetic tests at Beltsville were ended in November 1971. Some of the surviving parthenogenetic males were used in studies to establish organ weights. Of the four parthenogens still alive on November 1,

1971, one was 9 years, 8 months, and 5 days old; the second, 8 years old; the third, 5 years, 9 months, and 21 days old; and the fourth, 4 years, 8 months, and 25 days old. Records of the 46 parthenogenetic males that died of natural causes between November 1958 and November 1971 are as follows:

Number	Percent	Life span, years
2	(4.3)	< 1
12	(26.0)	< 2
21	(45.6)	< 3
24	(52.1)	< 4
32	(69.5)	< 5
39	(84.7)	< 6
42	(91.2)	< 7
46	(100.0)	< 10

The average lifespan of the 46 parthenogenetic males that died of natural causes was slightly less than 4 years. Three of the 50 parthenogens lived for over 9 years. The oldest of the four males still living appeared healthy November 1971.

Over 80 percent of the parthenogenetic males died before they were 6 years of age, despite the exceptionally long lifespan of three parthenogens. This high mortality among adult parthenogens at a relatively early age indicates that their lifespan is substantially shorter than that of normal BSW turkeys of the parthenogenetic strain.

Marsden and Olsen (49) showed that 11 of 15 (73.3 percent) of the BSW females on test were still living at 7 1/2 years of age. Seven of 11 BSW turkey females (63.6 percent) of another age group were still living at 9 1/2 years of age.

The 46 adult parthenogens that died of natural causes were autopsied. Seven deaths were attributed to chronic respiratory disorders, four to visceral leukosis and paralysis, three to enteritis, and two to tumors of the spleen. Each of the following conditions led to the death of one parthenogen: Infectious sinusitis, sour crop, chronic bacterial infection, nervous disorder, enlarged liver, tarsitis, nephritis, cervical abscess, excess fluid in body cavity, tumor of testis, and ruptured intestine. No specific pathological conditions were found at autopsy of 12 birds. Deaths of four other birds resulted from injuries or accidents. Post morten changes of two birds were too advanced to establish cause of death. No report was available on cause of death of one bird.

Although parthenogens strut and gobble like normal males, they have never been observed to mate or to make any serious attempt at mating. This is true even though the parthenogens at the time may be producing semen. Females, although inviting mating, are generally ignored and upon occasion are pecked by the male. All poults sired thus far by parthenogens have

been obtained through artificial insemination. On one occasion, a parthenogenetic male was placed in a pen with a virgin female. The hen proceeded to lay a clutch of eggs, all of which subsequently proved to be infertile. The parthenogenetic male sat on the nest and proceeded to incubate the eggs.

Parthenogens are particularly susceptible to perosis, which develops during the growing period. Perosis in this stock may be genetic since the five original families were discards from another program because they had this defect.

Parthenogens also appear to be quite susceptible to seizures. On being moved to new quarters or frightened, several birds have died with heart attacks.

The average growth rate of parthenogens is somewhat slower than that of normal BSW males, probably because of their high degree of inbreeding. BSW parthenogenetic males at maturity seldom weigh more than 18 to 20 pounds, although a few males have reached 24 pounds.

At Beltsville, in raising parthenogenetic poults, we kept them segregated from normal turkeys. Although the parthenogens are prone to fight among themselves, they are no match for normal males. Maintained in quarters where some degree of protection is provided, adult parthenogens have survived for several years.

POTENTIAL USES OF PARTHENOGENETIC MALES AND UNFERTILIZED EGGS

A special strain of BSW turkeys whose unfertilized eggs exhibit a high incidence of parthenogenesis has been developed at the Agricultural Research Center, Beltsville, Md. This special strain has been developed through 18 years of intensive selection of virgin hens for a high incidence of parthenogenetic embryos, in the presence of live fowl pox virus.

The frequency of parthenogenetic development at present is high enough to permit use of eggs of this stock for biological research on the embryo, lethal genes, mutations, skin grafting, organ transplants, and development of isogenic lines (80, 82).

Embryological studies.--Parthenogenetic development occurring in unfertilized oviducal eggs is atypical and unorganized. The delayed onset of development means that some developmental events (like formation of a normal single-layered blastoderm and gastrulation) occur, not in the hen's oviduct as is normal, but after the unfertilized eggs have been placed in the incubator. Stages in the formation of the blastoderm and gastrulation now can be obtained with comparative ease, in greater quantity, and without the necessity of sacrificing hens in order to obtain these stages.

Studies of lethal genes and mutations.--Various types of abnormalities appear frequently among parthenogenetic embryos that fail to hatch. Most normal turkey females appear to carry many mutant genes in the heterozygous form. In parthenogenetic development when diploidy is established, some

undesirable genes in homozygous form produce a great variety of abnormal conditions. These can be detected readily by examining embryos from unhatched eggs. If pedigreed, unfertilized eggs are used, the inheritance of such traits can be studied. Moreover, data of this type provide information not otherwise obtainable on the genotype of the virgin dam.

Skin-grafting and organ transplants.--Parthenogens and their dams make ideal combinations for testing principles of histocompatibility. The virgin dam has all the antigens possessed by her parthenogenetic son. Skin grafts as well as organ transplants from the parthenogenetic son to his dam should not be rejected because of an immune response. On the other hand, skin grafts and organ transplants from dam to son would be rejected since the parthenogen received only a sample one-half of the genes of his heterozygous dam.

Development of isogenetic lines.--Parthenogenetic sires are ideal birds for use in the rapid development of isogenetic lines. Turkey parthenogens may be homozygous at all or at most loci, making them not only highly inbred but also individuals that are essentially free of known embryonic lethals. A parthenogen, to be in existence, must of necessity be an individual almost completely free of embryonic lethals. At Beltsville, on several occasions, viable offspring was obtained from backcross matings of parthenogens to their dam (82, 84, 103). This type of foundation stock has been used in establishing inbred lines. Inbred sons (F_1) from backcross matings of a parthenogen to his dam have been successfully mated to their F_1 inbred sisters. Also, F_2 inbred sons have been mated successfully to their F_2 inbred sisters (83, 85). These matings are illustrated in the scheme of inbreeding shown in figure 14. Unfortunately, only about 20 percent of the parthenogens reaching maturity can be expected to produce even limited amounts of semen. Parthenogens that do produce semen, however, can sire normal, healthy male and female poults.

By using inbred sires (sons and grandsons of a parthenogenetic male by his own dam) on outbred hens from our high parthenogenetic incidence strain, Olsen (83) intensified in their virgin daughters the capacity for producing unfertilized eggs containing parthenogenetic embryos. During 1952-64, 68,879 unfertilized BSW turkey eggs were incubated of which 6,063 (8.7 percent) gave rise to parthenogenetic embryos and 305 (0.044 percent) to hatched parthenogens (62, 71). In contrast, during the 1971 testing season, 8,883 unfertilized turkey eggs were incubated of which 1,986 (22.4 percent) gave rise to embryos and 32 (0.36 percent) to hatched poults. Sixty-five young parthenogens hatched in the winter and spring of 1971 survived to December 1, 1971. Of these males 13 produced limited quantities of semen. Much of this rapid progress in our selective breeding program was attributed to an overall reduction of embryonic lethal genes in the parental stock through inbreeding. Theoretically, fewer lethals should allow more parthenogenetic embryos to form and to develop to the hatching stage.

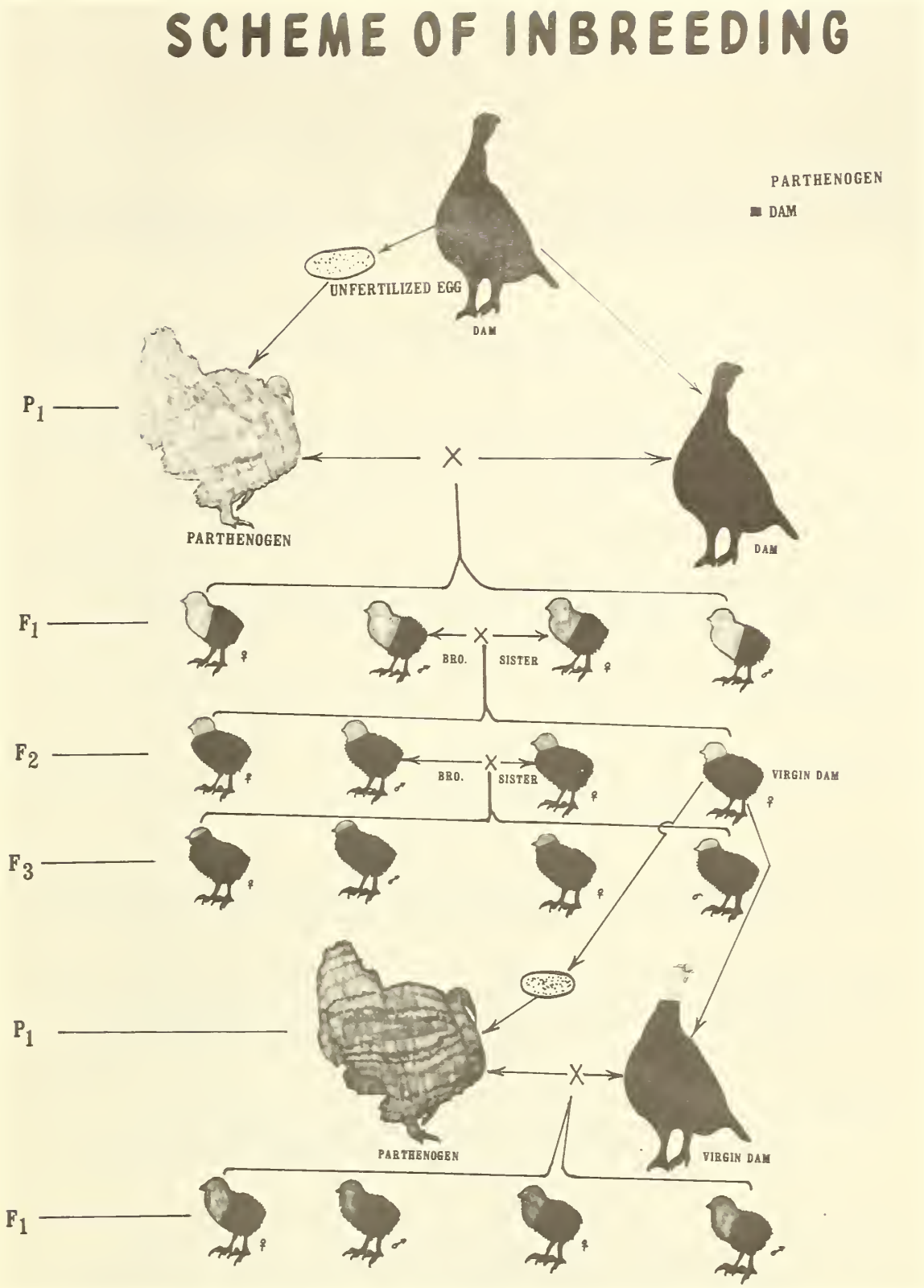

Figure 14.--Scheme of inbreeding.

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Appendix Table 15.--Incidence of parthenogenetic development in unfertilized eggs of Beltsville Small White turkeys, 1952-71, Beltsville, Md.

Year	Hens	Eggs	Parthenogenetic development	Embryos	Poults hatched
	Number	Number	Percent 1/	Percent 1/	Percent 2/
1952	29	934	16.9	0.2	--
1953	23	1,463	14.1	.2	--
1954	103	5,830	22.1	.7	2.3
1955	110	5,757	26.0	1.4	0
1956	126	6,003	30.5	4.2	3.2
1957	181	8,608	36.5	4.2	3.3
1958	214	8,045	40.7	9.0	2.8
1959	78	2,929	41.5	11.7	3.5
1960	60	2,815	39.8	9.8	5.4
1961	161	10,060	45.4	12.4	8.5
1962	120	8,519	46.0	12.6	8.8
1963	94	5,906	41.2	13.0	3.0
1964	79	2,910	40.9	10.4	4.9
1965	106	6,521	43.3	10.9	7.8
1966	14	728	46.2	12.1	6.8
1967	65	2,474	39.6	6.6	12.1
1968	168	7,933	40.9	8.4	4.4
1969	126	3,205	44.6	11.9	8.9
1970	136	6,248	45.4	15.3	8.9
1971	209	8,883	47.8	22.4	16.1
Total or average--	2,202	105,771	39.4	9.7	8.3

1/ Based on total eggs.
2/ Based on total embryos.

Appendix Table 16.--Incidence of parthenogenetic development in unfertilized eggs of Beltsville Small White turkeys, Dark Cornish chickens, and Japanese quail after 10 days' incubation, 1958-71, Beltsville, Md.

Year	Hens	Eggs	Parthenogenetic development	Embryos	Poults hatched
	Number	Number	Percent 1/	Percent 1/	Percent 2/
Old Beltsville Small White turkeys:					
1958-64	--	--	--	--	--
1965	60	1,614	45.2	5.3	11.6
1966	57	1,017	43.4	4.8	10.4
1967	49	1,887	31.3	2.2	24.0
1968	106	2,794	40.1	4.4	4.3
1969	128	4,429	43.1	4.6	12.3
1970	99	5,701	43.0	7.8	5.4
1971	118	4,344	45.4	11.2	5.1
Total or average	617	21,786	42.2	6.6	7.4
Dark Cornish chickens:					
1964	54	2,021	15.5	.1	--
1965	92	3,692	23.0	--	--
1966	88	3,419	25.4	--	16.5
1967	571	8,710	11.5	.05	--
1968	247	8,827	13.4	.09	--
Total or average	1,052	26,669	15.8	0.08	4.5
Japanese quail:					
1966	48	1,004	5.8	0	0
1967	150	1,165	0.0	0	0

1/ Based on total eggs.
2/ Based on total embryos.

Appendix Table 17.-- Incidence of parthenogenetic development in unfertilized eggs of turkeys, 1954-72, Beltsville, Md.

Location	Investigator	Breed or variety	Eggs	Parthenogenetic development	Embryos	Parthenogens hatched
		TURKEYS	Number	Number	Number	Number
ARC, Beltsville, Md.	M.W. Olsen	Beltsville Small White-	127,657	50,952	11,668	1,120
		Broad Breasted Large White	534	85	4	0
		New Jersey Buff	579	40	2	0
	P. Sarvella	Beltsville Small White-	1,882	700	330	6
Washington State University, Pullman.	I.L. Kosin	Washington State White-	367	29	2	1
		Beltsville Small White-	1,065	362	45	2
		Broad Breasted Bronze	1,141	47	0	0
University of California, Davis.	H. Abplanalp	Beltsville Small White-	1,067	314	24	3
Oregon State University, Corvallis.	J.A. Harper	do	389	26	0	0
Pennsylvania State University, State College.	E.G. Buss	do	4,486	1,960	486	62
Virginia Polytechnic Institute, Blacksburg.	A. Leighton, Jr.	do	3,543	549	10	3
University of Maine, Orono.	A. Mun	do	388	109	27	0
Texas A&M University, College Station.	R. Atkinson	Broad Breasted White	6,000	720	20	0
Loughry Agricultural College, Cookstown, Northern Ireland.	J.W. Young	Beltsville Small White-	(?)	--	9	1
Cornell University, Ithaca, N.Y.	S. E. Bloom	do	472	82	12	0
		CHICKENS				
ARC, Beltsville, Md.	M.W. Olsen	Dark Cornish	26,669	4,214	22	1
	P. Sarvella	do	33,376	15,692	378	10
		White Leghorn	4,089	163	0	1
Washington State University, Pullman.	I.L. Kosin	White Olympia	1,022	6	2	1

1/ Includes all unfertilized eggs exhibiting parthenogenetic development that could be detected by marcoscopic examination, including eggs showing only a limited, unorganized type of tissue growth as well as those in which recognizable embryos appeared.

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